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Study of the Morphological Characteristics of the Regenerating Suprapharyngeal Ganglion and Its Correlation with the Reemergence of Light Avoidance Response in Lumbricus Terrestris

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STUDY OF THE MORPHOLOGICAL CHARACTERISTICS OF THE REGENERATING
SUPRAPHARYNGEAL GANGLION AND ITS CORRELATION WITH THE
REEMERGENCE OF LIGHT AVOIDANCE RESPONSE
IN LUMBRICUS TERRESTRIS

by

Stephen Christopher Moore

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
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BIOGRAPHY

Stephen Christopher Moore was born July 19, 1951, in Peoria, Illinois.

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I. INTRODUCTION

Investigations of the nervous system of annelids began in the late 1800's with the work of Retzius (1892) and Hesse (1894). A comprehensive study of the nervous system of the earthworm, Lumbricus terrestris (L.) with physiological correlates was published by Hess (1924, 1925a,b). He reported that the suprapharyngeal ganglion controlled the light avoidance response in normal earthworms (negative phototropism), and that this response was reversed when the ganglion was removed (positive phototropism). A gradual return of the light avoidance response accompanied the regeneration of the suprapharyngeal ganglion and these two events were therefore correlated.

Throughout the 1900's most of the work concerning the central nervous system of annelids was confined to its effect on the regeneration of amputated caudal segments (Clark and Bonney, 1960; Clark and Clark, 1959; Clark and Evans, 1961; Clark and Ruston, 1963; Golding, 1965; Scully, 1964). Few investigations focused on the regeneration of central nervous tissue itself. One of the first studies in this area was by Goldfarb in 1909. He amputated the first four segments of the earthworm, Lumbricus terrestris, and subsequently removed the ventral nerve cord from the sixth to thirteenth segments to observe its effect on the regeneration of the suprapharyngeal ganglion. In 50% of the worms the ganglion regenerated and reestablished its connections with the ventral nerve cord.

In 1932, Schwartz extended the work of Goldfarb with an investigation of the origin of the cells which form the new ganglion and the rate in which regeneration is completed. His findings indicate that the neurons which form the regenerated suprapharyngeal ganglion are formed by a metamorphosis of connective tissue cells that infiltrate the ablated area. The external morphology of the new ganglion appears normal after six weeks. During this time, nerve trunks extending to the prostomium and normal connections with the circumpharyngeal connectives are formed.

No one has investigated the regeneration of the suprapharyngeal ganglion of Lumbricus terrestris using electron microscopy. Consequently, the main purpose of this study is: (1) to describe ultrastructurally, the stem cells, their metamorphosis, and their formation of functional neural elements in the regenerating ganglion; (2) to repeat the experiments of Hess and Schwartz to test their respective theories that the supra-pharyngeal ganglion is responsible for negative phototropic responses in earthworms and that connective tissue stem cells form the new ganglion cells; (3) to combine the data to determine whether the suprapharyngeal ganglion functions as the integrator of sensory information from epidermal photoreceptors and whether it is responsible for light avoidance responses seen in normal earthworms.

II. REVIEW OF THE RELATED LITERATURE

A. Introduction

The detailed anatomy of the suprapharyngeal ganglion of the earthworm, Lumbricus terrestris, has been described by Hess (1925). The ganglion is located in the dorsal aspect of the third segment, above the pharynx, within the coelomic cavity. It is connected to the ventral nerve cord through the subpharyngeal ganglion via the circumpharyngeal connectives. The prostomium is supplied by a pair of large nerve trunks which arise from the lateral aspect of the suprapharyngeal ganglion (fig. 1).

Ontogenetically both the "brain" and the ventral nerve cord develop from a teloblastic area of ectoderm (Pemmers, 1924; Schmidt, 1926). The neural strips are paired but fuse early in development so that the central nervous system is incompletely divided by a connective tissue septum. The nervous system is enclosed within a sheath which adjusts the length of the CNS during movements. This sheath is approximately 30 μ thick and is composed of collagen, circular and longitudinal muscle layers, interstitial cells, and vascular channels (Levi, et al, 1966).

B. Description of the Suprapharyngeal Ganglion Cellular Rind

Light microscopic studies of the suprapharyngeal ganglion have shown this structure to be relatively simple, lacking the internal division (lobes) and cellular diversity of higher classes of annelids such as the errant polychaetes (Bullock and Horridge, 1965). The adult ganglion can be divided into an outer rind containing the various cell bodies and an inner

core containing fiber tracts and the neuropile. The rind of the earthworm, Pheretima communissima, contains approximately 12,000 nerve cell bodies (Ogawa, 1939). The phylogenetically most advanced feature of the supra-pharyngeal ganglion are the "characteristic" cells described by Ogawa, which are interneurons located in its anteriodorsal aspect. These cells are heteropolar-multipolar, and unlike their equivalents in vertebrates, in which these cells form the universal type of interneuron or motoneuron, they are only found in the higher classes of invertebrates. They are small neurons with short processes that extend into a plexus at the surface of the neuropile, and they comprise 50% of the nerve cell bodies.

Two other types of interneurons, small and large, can also be found in the cellular rind. The small interneurons are unipolar cells that have long axons devoid of collaterals. Their processes project to the neuropile of both the suprapharyngeal and subpharyngeal ganglia. The large interneurons are located in the ventral portion of the cell rind. These neurons send both contralateral and ipsilateral processes to the neuropile of the suprapharyngeal and subpharyngeal ganglia.

The cell rind also contains unipolar motoneurons. These cells are differentiated from vertebrate motoneurons in that their axons contain many collaterals which form extensive synapses in the neuropile.

Levi, et al (1966), working on the subpharyngeal ganglion of Lumbricus, found three types of glial cells, differentiated to some extent by their cytoplasmic characteristics which vary according to their location. The three types of cells are: (1) cells at the surface of the ganglion which separate the neurons from the collagen and vessels of the nerve cord

sheath. Using the pattern of sequential labeling in autoradiography and the staining characteristics of an intravital dye (trypan blue), Levi suggests that these cells not only lend physical support to the nervous system but regulate the diffusion or active transport of material from the sheath to the neurons as well. These cells contain a large number of multivesicular bodies and electron dense vesicles (0.1 to 3 μ in diameter). (2) Glia that separate the ganglion cells of the rind from the neuropile of the core. They contain the same cytoplasmic organelles as those in the first group. (3) Cells associated with the nerve cell bodies and their processes. These glial elements are similar to the oligodendrocytes and satellite cells found in vertebrates. They form the sheath of lamellated cell processes that surround the parikaryon and its processes. These cells also form the "loose" myelin of the giant nerve fibers located in the ventral nerve cord. The cytoplasm of these glia is filled with a large number of fibrils approximately 50Å in diameter.

C. Description of the Suprapharyngeal Ganglion Neuropile

The fibers within the suprapharyngeal ganglion include: (1) afferents from the epidermis of the prostomium; also from the first segment (Hess, 1925a), or from the first and second segments (Ogawa, 1939); (2) afferents from the prostomium which pass through the suprapharyngeal ganglion and synapse in the subpharyngeal ganglion; (3) afferents from the wall of the buccal cavity; (4) efferents from large cells in the dorsal aspect of the ganglion; (5) efferents originating in the subpharyngeal ganglion which run to the suprapharyngeal ganglion or pass through it and run to muscles of

the prostomium (Bullock and Horridge, 1965).

The neuropile of the suprapharyngeal ganglion is divided into anterior, middle, and posterior regions. The anterior neuropile contains the fibers of the "characteristic" cells. The middle neuropile contains the fibers of afferent neurons from the epidermal sense cells and other sense organs. The posterior neuropile contains the processes of the large interneurons as they ascend from or descend into the subpharyngeal ganglion (Bullock and Horridge, 1965).

Myhrberg (1972), working on the suprapharyngeal ganglion of Lumbricus, has classified the various types of vesicles found within the neuropile. Gluteraldehyde and osmium tetroxide fixation reveals four categories: (1) pale, agranular vesicles (300-500Å in diameter) are the most prevalent and are thought to contain acetylcholine (Rosenbluth, 1972). (2) Halo vesicles (700-1200Å) contain an electron dense core separated from a limiting membrane by a clear electron translucent area. They contain the catecholamines noraadrenalin or dopamine. (3) Dark, granular vesicles (600-900Å) contain the monoamines noradrenalin, dopamine or 5 hydroxy-tryptamine. (4) Large neurosecretory vesicles (1200-3500Å) contain a homogenous material in one of two possible states: (a) electron translucent (pale) granules which possibly contain catecholamines; (b) electron dense (osmiophilic or dark) granules which probably contain one of three known earthworm neurohormones (vasopressin, oxytocin, or chromatophorotropin) (Scharrer and Brown, 1961).

Fluorescence studies on polychaetes (Nephtys) have identified the green fluorsence characteristics of noradrenalin or dopamine as the major

neurotransmitter in the suprapharyngeal ganglion. These transmitters are associated with the sensory system. Since many of the neurons arising from the epithelium of the prostomium project to the suprapharyngeal ganglion where they are involved in multineuronal, multisynaptic pathways, these fibers function as sensory integrators and not simply as reflex arcs (Clark, 1967).

D. Physiological Correlates to the Suprapharyngeal Ganglion

Physiologically, the suprapharyngeal ganglion has been associated with neurosecretion (Clark, 1956a, 1959, 1967; Herlant-Meewis, 1955; Hubl, 1953; Scharrer, 1937; Scharrer and Brown, 1961; Scharrer and Scharrer, 1954; Schmid, 1947), reproduction (Clark, 1956b, 1965, 1967; Durchon, 1962; Herlant-Meewis, 1956, 1959; Hubl, 1953, 1956; Roux, 1953), regeneration of amputated caudal segments (Clark and Bonney, 1960; Clark and Clark, 1959; Clark and Evans, 1961; Clark and Ruston, 1963; Golding, 1965; Hubl, 1953, 1956; Scully, 1964), responses to sensory stimuli such as light (Hess, 1924; Howell, 1939), touch (Laverack, 1960) and pH (Laverack, 1961).

Neurosecretion is a primary function of the suprapharyngeal ganglion of annelids. Various authors have estimated that 50% to 100% of the ganglion cells in polychaetes are capable of producing and releasing neurohormones (Scharrer, 1937; Clark, 1959). Schmid (1947), found that a large number of cells in the suprapharyngeal ganglion of Lumbricus can be induced to secrete granular material when novocaine or epinephrine is injected into the coelom.

In general, neurosecretion in *Oligochaetes* has been correlated with:

- (1) maturation of gametes;
- (2) development of somatic sex characteristics;
- (3) regeneration; and
- (4) color change.

Four different types of secretory cells have been found in the ganglia of the earthworm, Eisenia foetida (Herlant-Meewis, 1955, 1956) and several species of Lumbricus and Allolobophora (Hubl, 1953, 1956). According to Herlant-Meewis they are the acidophilic or a-cells, the basophilic or b-cells, the large and medium-sized cells (three groups within the suprapharyngeal ganglion), and the subpharyngeal neurosecretory cells.

The a-cells are located in the posteriolateral area of the suprapharyngeal ganglion and are thought to control somatic sex characteristics. The release of their hormones in the spring and summer promotes sexual activity. Roux (1953) found that the number of a-cells diminishes after gonadectomy. Similarly, the ablation of the suprapharyngeal ganglion blocks egg-laying in the Lumbriciden until the a-cells regenerate. This takes approximately 90 days.

The b-cells and the subpharyngeal neurosecretory cells control regeneration of amputated tail segments. Hubl (1953, 1956), working on Lumbricus, found that the suprapharyngeal ganglion is required for regeneration during a critical period of one or two days following amputation. Golding (1965) performed a series of grafting experiments on the polychaete Nereis diversicolor. In one of these experiments, he grafted caudal segments from amputated donors, which had been regenerating for a period of one to four weeks, onto intact hosts. He then amputated caudal segments from the host. He found that the rate and number of regenerated segments of the

grafts were independent of that of the host. Clark and Scully (1964) suggest that the suprapharyngeal ganglion produces the "regeneration" hormone at a constant rate but that the amputation alters the sensitivity of the tissue to the hormone. The rate and degree of regeneration depends on the changing response threshold of the tissue to the "regeneration" hormone.

In the early 1900's it was known that the suprapharyngeal ganglion ablated worm could regenerate a new ganglion, but little was known about the process or the stem cells until the experiments of Schwartz (1932). Using light microscopy, he found that the new ganglion cells are formed from invading connective tissue cells that replace the excised ganglion. The first stage of regeneration involves an infiltration of the site of lesion by connective tissue and muscle cells dorsolateral to the cut connectives. The metamorphosis of a functional nerve cell takes approximately 13 days. During this time the nuclear chromatin of the fibroblast decreases while the concentration of cytoplasmic chromatin (Nissl substance) increases. A nucleolus is formed eight days after ablation and by the tenth day a pseudopodial process develops from the cell cytoplasm. This process becomes a functional axon at 13 days, signifying the formation of a unipolar nerve. Until the ninth day of metamorphosis the "transitional" cell can divide, but no mitosis is observed after this day. It appears, therefore, that annelid nerve cells, like those of vertebrates, are incapable of division. Some of the connective tissue cells that do not differentiate form part of the nerve cord sheath. In general, the ganglion appears

cytologically functional three weeks after ablation. At this time the circumpharyngeal connectives and prostomial nerves have synapsed with the newly formed ganglion cells.

No specific cytological changes could be observed in the nerve cells of the subpharyngeal ganglion which might act as a stimulus for the cellular metamorphosis. Schwartz therefore concluded that surgical trauma acts as a general stimulus.

A significant amount of work has been done on the phototropic responses of normal and "decerebrate" earthworms (Hess, 1922, 1924; Howell, 1939). Hess (1924) found that normal earthworms were negatively phototropic to a moderate light stimulus. After the suprapharyngeal ganglion had been removed the worms became positively phototropic for a period of 14 to 24 days. Nevertheless, during this period the ablated worms gradually exhibited an increased number of negative responses which, during the ten day interval described above, became entirely negative.

The cells that are responsible for negative phototropic responses have been studied with light (Hess, 1922, 1923, 1925; Howell, 1939) and electron microscopy (Röhlich, 1970). Photoreception in the earthworm is accomplished by scattered single cells throughout the epidermis. The prostomium and anterior segments have the largest concentration of photoreceptors while the caudal segments have a higher concentration than the middle segments. These cells are also found under the sheath of the suprapharyngeal ganglion and clustered as enlargements along the prostomial nerves and some of the caudal segmental nerves (Hess, 1925).

Light microscopy of the cytoplasm reveals many neurofibrils and a large refractile body called the "retinelle" (Hess, 1925). The retinelle is probably the same inclusion described by Röhlich (1970) in ultrastructural studies as the "phaosome". This structure fills a large area in the cytoplasm of the photoreceptor and consists of many microvilli and a few cilia. The microvilli contain visual pigment and are thought to produce the generator potential within the neuron.

Hess (1925) found that the fibers of the photoreceptors and other receptors (mechanoreceptors and "supporting" cells that are chemoreceptors) project to two main areas. One branch of the axon extends into the CNS through a segmental nerve while a second branch projects to the subepidermal nerve plexus. This plexus is non-segmental and participates in neuromuscular reflexes that are independent of the CNS. The existence of a dual nervous system (subepidermal plexus and the CNS) whose fibers intermingle and yet where various receptor-effector responses are autonomous of central control, characterizes the phylogenetic transition in the nervous system of the earthworm from the plexus of lower animals to the synapse found within the well defined central nervous system of higher animals (Hess, 1925).

III. MATERIALS AND METHODS

Specimens of Lumbricus terrestris were procured from local bait shops. They were kept in large plastic containers containing sterilized potting soil and moistened leaves at a temperature of 12°C. ($\pm 1^\circ\text{C}.$)

Two groups of worms were used to collect data on phototropic responses. Each of the two groups was exposed to a different light intensity during the test (56 or 14 foot candles). The worms were dark adapted for at least 30 minutes and were then placed on a large sheet of dark green blotting paper (40 by 45 cm.) that was kept moist with distilled water. Each worm was illuminated vertically from a height of 50 cm. (Hadek and Rosen, 1973) using a microscope illuminator with a 30 watt G.E. light bulb. Each test consisted of ten trials with a 30 second illumination and a 60 second intertrial period. While testing, the worms were manipulated with wooden probes.

Earthworms from which the suprapharyngeal ganglion was removed were anesthetized in 5% urethane for approximately ten minutes (until spontaneous contractions ceased). Surgical equipment was washed in 35% alcohol and surgical gloves were worn during the procedure. The dorsal aspect of the third segment was opened with a single edge razor blade. The suprapharyngeal ganglion was located using a dissecting microscope. It was lifted away from the gut as the circumpharyngeal connectives were cut close to the ventral nerve cord. The worms were quickly washed with distilled water to accelerate recovery. For nine days they were housed in plastic boxes (13x8x5cm.) containing moistened filter paper, after which they were

transferred to boxes containing sterilized soil and moistened leaves.

A sham operation was performed on a group of five worms to determine the effect of surgical trauma on the phototropic responses of non-ablated earthworms. The worms were anesthetized as above. An incision was made in the dorsal aspect of the third segment and the ganglion was located but not removed. Subsequently the worms were treated and tested in the same way as the "decerebrate" earthworms.

Data on the phototropic responses of earthworms with excised supra-pharyngeal ganglia was collected every three days for a period of 27 days (until a definite pattern of response was established). Two specimens were killed every three days and processed for light microscopy. Specimens of 12, 18, 26, and 34 days postoperative were killed and processed for electron microscopy.

The tissue processed for light microscopy was fixed in a 10% buffered formalin solution for 24 hours:

- 100ml. of 38% formaldehyde solution
- 4gm. acid sodium phosphate monohydrate
- 6.5gm. anhydrous disodium phosphate
- 900ml. distilled water

The 3 and 12 day tissue samples were mordanted for 24 hours in Weigert's primary mordant following the initial fixation:

- 5mg. potassium dichromate
- 2gm. chromium fluoride
- 100ml. distilled water

The tissue was then dehydrated in a series of ethyl alcohol baths of increasing strength, cleared in benzene, and infiltrated and embedded in paraffin. Serial sections were cut at a thickness of 10 μ and stained for one hour according to Nocht's azure-eosin method for Nissl substance using thionin and eosin B at a pH=4.0. The tissue mordanted in the dichromate solution was stained according to the same procedure for a generalized chromaffin reaction (Lillie, 1954). The sections were dehydrated in acetone, cleared in xylene, and mounted. All micrographs were taken from specimens stained with thionin and eosin B on a Zeiss "Ultraphot".

Processing for transmission electron microscopy involved fixation in 2.5% phosphate-buffered gluteraldehyde (pH=7.4) followed by 1% phosphate-buffered osmium tetroxide at a temperature of 5°C. (Millonig, 1961). The tissue was dehydrated in a graded ethyl alcohol series to absolute ethyl alcohol (5°C.), cleared in propylene oxide (room temperature), and embedded in Epon 812 (Luft, 1961). The tissue was sectioned with glass knives on an AO OMU2 Reichert ultramicrotome. Sections were mounted on 300 mesh grids and stained with 5% uranyl acetate (Swift and Rasch, 1958). They were examined on an RCA-EMU-3F2 electron microscope.

Studies of the normal suprapharyngeal ganglion were undertaken using scanning electron microscopy. Earthworms were anesthetized in 5% urethane for 15 minutes and then immediately fixed in toto in a 2.5% gluteraldehyde/14% sucrose solution for 60 minutes (Smith, et al, 1973). The nervous system was then dissected out and fixed for an additional 30 minutes. The tissue was dehydrated in a graded alcohol series to absolute alcohol, followed by absolute amyl acetate. The dehydrated suprapharyngeal

ganglion was freeze-dried in a critical point drying apparatus. It was then carbon and gold coated and examined with the Mini-Sem scanning electron microscope.

IV. RESULTS

A. Light Microscopy

The results of the light microscopic investigation concur to a large extent with the results of Schwartz (1932). The area of the ablated ganglion was quickly filled with cells that resembled fibroblasts. By the ninth day the presumptive neural cells had undergone metamorphosis, and by the fifteenth they had formed unipolar neurons. Most of the ganglion had regenerated and formed its connections with the prostomial nerves at 21 days postoperative. The normal suprapharyngeal ganglion (fig. 2) presented the characteristic cell bodies in the dorsal and lateral aspects of the rind, surrounding the neuropile. Thionin stained the ribonucleoprotein (RNP) particles within the cytoplasm (granular endoplasmic reticulum) and within the nucleolus of the nerve cell bodies. Groups of connective tissue associated with bundles of muscle fibers filled the area dorsal to the gut and ventral to the suprapharyngeal ganglion. Muscle from the body wall surrounded the dorsal and lateral regions of the ganglion. Eosin B stained the cytoplasm of the muscle fibers and the processes within the neuropile a bright pink.

Three days after the suprapharyngeal ganglion was excised, the area was filled with many newly formed vessels, muscle and connective tissue fibers, and extracellular cytoplasmic vesicles. Potassium dichromate fixation revealed a distinct chromaffin reaction within these free vesicles after they were stained with thionin (figs. 3,4). In an attempt to locate a possible source of the vesicles the subpharyngeal ganglion was also

stained. The cell bodies of many of the neurons showed a strong chromaffin reaction as well as a degree of vacuolization, characteristic of a release of their cellular product (fig. 5). Other cell bodies within the area apparently had not entered the secretory cycle revealed by this reaction.

No trace of hemorrhage was found in the region of the ablation, and the cut connectives were smoothly rounded. Hypertrophy and hyperplasia of the muscle and connective tissue had occurred in the areas dorsolateral and ventral to the excised ganglion.

Nine days postoperative revealed a dense infiltration of connective tissue into the region of the ablated ganglion, stretching between the two cut connectives. The number of "connective tissue cells" had increased but they were confined to this region. A large portion of the ablated area was completely denuded—no longer loosely filled with the random muscle and connective tissue fibers that had characterized the three day postoperative tissue (fig. 6). Dorsolaterally, the boundary of this denuded area was formed by hyperplastic muscle from the body wall. The "dense" connective tissue between the cut connectives originated from the area ventral and dorsolateral to the excised ganglion. The nine day tissue also showed that the connectives were growing dorsomedially, and had advanced considerably by this time.

Many of the fibroblast-like cells had begun to acquire the features of neurons, undergoing both cytoplasmic and nuclear metamorphosis (arrows). Most of these "transitional" cells were found in the area dorsomedial to the growing connectives. Thionin-stained granular endoplasmic reticulum appeared in the cytoplasm. The nuclear chromatin was dispersed to the

periphery, except for a prominent nucleolus that also stained with thionin. The external morphology of the cell and its scanty cytoplasm prevented its classification as a true nerve cell—the nucleus was not yet rounded and the cytoplasm was still elongated, characteristic of a fibroblast. These cells were more numerous in the 15 day postoperative tissue (fig. 10).

Potassium dichromate fixation on the twelfth postoperative day failed to show any chromaffin reaction in the ablated area. The number of secretory cells in the subpharyngeal ganglion containing catecholamines had also been reduced to approximately two. This is close to the normal number described by Schmid (1946). The most characteristic feature of the 12 day tissue was the hypertrophy of muscle fibers in the region ventral to the ablated ganglion (fig. 7). While the stroma of the ventral muscle mass had connected with the circumpharyngeal connectives and the connective tissue between them at nine days, the 12 day postoperative muscle appeared to expand dorsally into the area that had been completely denuded at nine days. The muscle of the body wall dorsal to the ablated area had not changed significantly at this time.

The fifteenth postoperative day provided an important focal point in the regeneration process. At this time, the metamorphosing tissue, composed of ganglion cells and a central neuropile, had acquired many of the characteristics of a normal suprapharyngeal ganglion (fig. 8). It was bilobed and had rejoined the circumpharyngeal connectives. This tissue was also important because a large population of three distinctive cell types, fibroblast-like cells, "transitional" cells, and neurons, were all present in the same area.

The largest concentration of "fibroblasts" was found dorsal to the growing ganglion and circumpharyngeal connectives. These cells had small, eccentric nuclei containing several clumps of chromatin situated in the center. The scanty, elongated cytoplasm was stained pink with eosin B (fig. 9).

The transitional cells and true nerve cells were centrally located, surrounded by the fibroblasts. One group was found near the fibers joining the connectives to the presumptive suprapharyngeal ganglion. Another large population was found in the area ventral to the developing neuropile. The transitional cells were similar to those in the nine day tissue, although the cytoplasm in many of them had increased in size and in concentration of granular endoplasmic reticulum (fig. 10). The important feature was that the nucleus was eccentric or oval and the cytoplasm was still elongated.

The true nerve cells had large, rounded nuclei with a prominent nucleolus. The remainder of the nucleoplasm contained a small amount of chromatin, dispersed in the periphery near the nuclear membrane. The amount of cytoplasmic RNP particles was greater than in the transitional cells, and the majority of the particles were concentrated in the axonal end of the neuron (fig. 11). Most of the nerves were unipolar but their size was variable.

The characteristic feature of the 30 day postoperative tissue was a very large concentration of thionin-stained granular endoplasmic reticulum in the unipolar nerve cell bodies (fig. 12). The cytoplasm of these cells had increased noticeably in size and appeared to be completely filled with

RNP particles. Schwartz (1932) noticed this same phenomenon in tissue older than ten days postoperative but by 21 days he found that the concentration had returned to normal. The tissue in this investigation appeared normal at 34 days postoperative.

The 84 day suprapharyngeal ganglion was identical to the normal in size, shape, and the number of cell bodies (fig. 13). The unipolar nerves also appeared normal, but there were still conspicuous areas of darkly stained granules throughout the cytoplasm (fig. 14).

B. Scanning Electron Microscopy

No investigations of the suprapharyngeal ganglion using scanning electron microscopy were found in the literature and therefore a study was undertaken to describe the surface structure of the ganglion and other components of the nervous system. A low power photomicrograph of the CNS of Lumbricus showed that the entire nervous system was surrounded by a nerve cord sheath, and the sheath, in turn, was covered by a layer of peritoneum (fig. 15). This feature resulted in greater sheath dimensions than had been described in earlier light and electron microscopic measurements (25-40 μ). It also appeared to be composed of several sheets of tissue, presumably muscle and connective tissue (Levi, et al, 1966), although this could not be determined in higher magnification photomicrographs. A small section of the sheath was cut away in figure 15 to reveal the anteriodorsal surface of the suprapharyngeal ganglion.

The circumpharyngeal connectives (extending to the subpharyngeal ganglion) and a portion of the ventral nerve cord was also shown. The

center of the sheath covering the ventral nerve cord was noticeably indented. This area overlies the incomplete connective tissue septum that was formed when the two halves of the embryonic nerve cord fused early in development (Penners, 1924; Schmidt, 1926). Several large nerve trunks extending from the segmental ganglia were also found.

C. Transmission Electron Microscopy

Results of an ultrastructural study of cells within the regenerating suprapharyngeal ganglion concur with the light microscopic data presented earlier. Cytological changes in the fibroblast-like cells within the developing ganglion indicated a specific metamorphosis from "connective tissue" to nerve. Following a general infiltration by muscle at 12 days, two specific stages of cytoplasmic development occurred within the "fibroblast" cells. The first stage was found in the 26 day postoperative tissue in which the cytoplasm was filled with so many RNP particles that other organelles were difficult or impossible to find. This coincided with the light microscopic data of the 30 day tissue and was indicative of the cellular synthesis of protein (neurohormones, axoplasmic constituents, etc.) or transmitter substances.

The second stage involved the 34 day postoperative tissue. The number of RNP particles had returned to normal levels, but an elaborate matrix of agranular endoplasmic reticulum had taken its place. Cellular metabolism had apparently turned from the synthesis of materials to their packaging for transport. It was evident that a majority of ganglion cells at these two stages of development did not yet have the cytoplasmic

characteristics of "normal" neurons.

A normal nerve cell body within the suprapharyngeal ganglion is shown in figure 16. The most prominent feature was the large number of vesicles within the cytoplasm. They ranged in diameter from 1200 to 2600Å; relatively large vesicles that were probably neurosecretory (Myhrberg, 1972). Furthermore, there were two populations of vesicles. Both groups contained material that was enveloped by a single limiting membrane, but one population contained electron dense material (probably peptidergic) while the second contained a relatively electron translucent substance (probably catecholamine according to Myhrberg).

A relatively large (1.4μ) multi-vesicular body composed of separate vesicles enclosed within a single limiting membrane was also discovered. Bargmann (1958) also found neurosecretory material that had coalesced into a large cellular inclusion.

A striking cytoplasmic feature was the predominance of free RNP particles over organized rough endoplasmic reticulum. Aside from this only a few mitochondria and degeneration products were found. Surprisingly, there was no agranular endoplasmic reticulum (Golgi apparatus).

The cell membrane gave evidence of two deep invaginations. This feature was termed the trophospongium by Malholtra (1957) and is formed by the processes of glial cells. The numerous membranes, filaments, and a desmosome in this region are also indicative of glia.

A glial cell was found in the suprapharyngeal ganglion near the nerve cord sheath in an area containing muscle, an amorphous ground substance, and vascular lacunae (fig. 17). The central portion of the nucleus

contained several large clumps of chromatin, while smaller, homogenous areas were distributed along the nuclear membrane. The cytoplasm was filled with mitochondria, vacuoles, and granules that resembled free ribosomes. The glial membrane enveloped a single nerve fiber and in this area a large number of filaments were cut in longitudinal and cross section.

The neuropile of the suprapharyngeal ganglion (fig. 18) contained a large concentration of clear "synaptic" vesicles approximately 300 to 500Å in diameter. These axons are presumably cholinergic (Rosenbluth, 1972). A number of dense core "halo" vesicles (900-1500Å) were also found, and Myhrberg stated that these contain catecholamines. Several dark vesicles (250Å) without limiting membranes were observed.

Two different populations of vesicles were often found in a single axon terminal. The most noticeable combinations were clear and halo vesicles or clear and neurosecretory vesicles within the same bouton. This phenomenon has been described in other annelids but has not been found in vertebrates.

Although the presynaptic terminal contained the typical vesicles and mitochondria, no structures such as subsynaptic webs or cisterns, or presynaptic and postsynaptic membrane thickenings often reported in vertebrate boutons (Pappas and Papurra, 1972) were observed in this tissue. Conspicuous bands of filaments, however, were seen in many of the axon terminals.

The ultrastructural study of the 12 day postoperative tissue revealed muscle and "connective tissue" infiltration in the region of the regenerating ganglion. During the gross dissection of the nervous system,

this tissue was enveloped in a nerve cord sheath that was continuous with the circumpharyngeal connectives. Light microscopic data showed that this tissue originated from the muscle ventral to the ablated ganglion (fig. 7).

The muscle resembled that of the body wall except for a number of fibers that had undergone profound degenerative changes (fig. 19). In this figure, a normal muscle bundle was adjacent to one in which degeneration products had formed myelin figures. The t-tubular system was scanty and incomplete, and larger vacuoles were developing throughout the sarcoplasm, especially near the myelin figures. The periodicity of the thick filaments was also erratic, and their diameter was greater than that in the normal muscle. This sequence of cellular proliferation followed by cell death was characteristic of each stage of ganglion regeneration.

Muscle hypertrophy was accompanied by "fibroblast" infiltration and metamorphosis. At 12 days postoperative, the nuclear chromatin of many fibroblasts had diminished (fig. 20). The nucleus of this cell was still elongated; the cytoplasm had sparse populations of mitochondria and dark, pleomorphic inclusions. The characteristic feature of the cytoplasm, however, was a complex arrangement of filaments. Figure 20 shows a typical longitudinal array close to the nucleus. In the distal portion of the cytoplasm the filaments formed a spiral or "fingerprint" configuration. The cytoplasm had also accumulated a number of free RNP particles and a larger population of ribosomes in rosette formations. The fibroblasts and their processes were interspersed between muscle bundles.

Many of the cells of the 18 day postoperative tissue had nuclear and cytoplasmic features characteristic of normal neurons. The nuclei were

rounded and contained sparse amounts of chromatin, except for a prominent nucleolus (figs. 21,22). For the most part, cytoplasmic organelles appeared "normal". RNP particles consisted of rosettes or free ribosomes, and for the first time since the beginning of metamorphosis, well organized rough endoplasmic reticulum was formed. However, the ribosomes were aligned along membranous cisterns that were both irregular in shape and abnormally wide (3500Å in fig. 28). Agranular endoplasmic reticulum was found among a small population of osmiophilic vesicles. The cell was therefore capable of synthesizing and packaging neurosecretory products. Small, rounded mitochondria were also numerous but the most striking feature was the large population of filaments found throughout the cytoplasm (fig. 21).

The 18 day regenerated ganglion also had a well developed neuropile, although the boutons lacked much of the synaptic surface found within the normal neuropile (fig. 23). Even at this early stage of development, single axons often contained different types of vesicles. Although the neurosecretory and "halo" vesicles were similar to those in the normal ganglion, two populations of clear "synaptic" vesicles were present, based upon size.

The 26 day postoperative tissue presented a unique example of cytoplasmic transformation and specialization. Compared to the relatively normal, homologous cytoplasm of the 18 day tissue, the 26 day cell was filled with many RNP particles, primarily in the rosette configuration or as free ribosomes. There were, however, a few formations of rough endoplasmic reticulum with normally shaped cisterns (figs. 24,25). The cell had apparently entered a stage of intense protein synthesis. A few mitochondria were also observed and the filaments that were first found in the 12 day

fibroblast had expanded into a system that invested the area around the nucleus (fig. 24). The filaments were oriented along the longitudinal axis of the cell and comprised the second largest category of cytoplasmic organelles after the RNP particles. The nuclei were generally rounded and contained a large nucleolus, although some of them still had fibroblast characteristics. The rest of the nucleoplasm contained a moderate amount of chromatin, with larger clumps found near the nuclear membrane.

The cells within the developing rind were densely packed while those in the outer region of the suprapharyngeal ganglion were embedded in an amorphous ground substance that contained many degeneration products (fig. 31). Cell death was still a prominent feature of the 26 day tissue. Although Schwartz reported glial formation that accompanied the connective tissue metamorphosis, none of their processes were found adjacent to the nerve perikaryon.

The 34 day ganglion cell had been functionally transformed from synthesis to packaging of neurosecretory substances. The cytoplasm was filled with an elaborate system of agranular endoplasmic reticulum, composed of parallel rows of double-walled cisterns ranging in number from 3 to 15 or more. The reticulum followed tortuous paths throughout the cytoplasm and its peripheral areas were swollen with an electron dense material. In one region of figure 26 the reticulum was fragmented into elementary vesicles composed of osmiophilic particles surrounded by membranes derived from the cisterns (arrows). Larger, electron dense vesicles with a well defined limiting membrane were found throughout the cytoplasm. In general, the agranular endoplasmic reticulum resembled a highly active form of the

characteristic vertebrate Golgi apparatus.

The cytoplasm was also filled with numerous small, rounded vacuoles. Many of them had ribosomes aligned along their membranes. The few that resembled normal rough endoplasmic reticulum were found adjacent to the Golgi apparatus. Free ribosomes were often joined by a thin filament of m-RNA to form a polysome. The cytoplasm also contained several large osmiophilic inclusions, but their origin and function was unknown.

Schwartz (1932) discovered that the normal burrowing activity of worms was lost after decerebration. He theorized that the prostomial nerves had not formed functional synapses within the suprapharyngeal ganglion, although the nerves had joined it at 21 days. One of the large prostomial nerve trunks was found within the 34 day ganglion near the nerve cord sheath, and a small, terminal branch of it was discovered deep within the cord sheath before it synapsed with superficial ganglion cells (fig. 27). The entire nerve was enveloped by collagen and a muscle bundle of the sheath, while individual neurons were surrounded by glial investments. Glial processes also separated the nerve fibers from the collagen of the sheath.

The boutons of the neurons contained a large concentration of clear (cholinergic) and dark "halo" (catecholamine) vesicles. Many mitochondria were also found within the axons.

D. Phototropic Response Studies

Experiments were conducted to quantify phototropic responses of earthworms. The tests involved the illumination of normal and supra-pharyngeal ganglion ablated worms with two different light intensities as changes in response patterns were recorded. The worms were placed on moistened blotting paper and surrounded by four wooden blocks which served as barriers. They were illuminated for 30 seconds/trial, although this interval had to be increased for the ganglion ablated worms in order to verify the responses. Four illumination patterns were used during a single testing period (10 trials) to insure a complete repertoire of conditions: (1) the anterior segments of the worm were illuminated; (2) the posterior segments were illuminated; (3) the worm was illuminated as it faced a barrier; (4) the worm itself was not illuminated but a beam of light was directed toward an area adjacent to it.

Normal earthworms elicited a strong avoidance response (negative phototropism) when illuminated with a light source of moderate intensity regardless of its position (table 1). The anterior segments were raised for approximately 5 to 15 seconds and then the "startle" reflex was completed with a series of peristaltic muscle contractions along the entire length of the body. Illumination of the anterior segments evoked the strongest and fastest response. It rarely took more than 20 seconds for the worm to crawl forward or pull itself back and away from the beam of light. When the posterior segments were illuminated the worm raised and turned its anterior segments toward the light before it pulled away.

A normal earthworm never entered a beam of light that was placed by its side. This condition always evoked a strong avoidance response. The longest latency period before a response occurred when the anterior segments were placed against an illuminated barrier. The worm usually crawled forward to the barrier, touched it with its prostomium several times, and then attempted to go over or under it. Unsuccessful at these attempts the worm would withdraw by pulling itself away until it could change direction and crawl from the area. This frequently took the entire 30 second illumination period.

The first group of suprapharyngeal ganglion ablated earthworms were tested with a moderate light intensity of 56 foot candles (table 1). In general, the spontaneous activity of these worms was greater than normal. This group also exhibited a unique form of locomotion that had not been reported in any previous investigation. For the first nine postoperative days the worms displayed "corkscrew" movements. Instead of the peristaltic waves of contraction, first within the circular muscle and then within the longitudinal, which lengthen and shorten the segments and provide movement for the normal worm, the decerebrate animals exhibited winding movements similar to those of a snake. The normal dorsal-ventral relationship was also lost so that locomotion was accompanied by a 360° rotation of the entire body. The worm then crawled as frequently on its dorsal surface as on its ventral.

This bizarre pattern of locomotion was ineffective in providing directional movements. To compound the problem, the muscles within the anterior and posterior segments frequently contracted simultaneously so that there was no net movement, even though there was a high level of

uncoordinated activity. Obviously, the response time after illumination was greatly increased. If the 30 second illumination/trial had been observed, the number of advances (positive phototropism) would have been much greater for the first 9 days postoperative. This strict time limit was not observed because of the major difference in response patterns before and during illumination. Before a test, the worm exhibited an increased spontaneous activity that included slow, undulating movements and elevation of the anterior segments. When the worm was illuminated, however, it thrashed about wildly and its body often coiled upon itself, in what appeared to be an attempt to escape. It was found that an extension to a 70 second illumination provided enough time to determine a response.

For both the normal and postoperative worms, a negative response was recorded when a worm moved out of a beam of light or away from an illuminated barrier. A positive response was recorded if a worm moved into a beam of light or remained at an illuminated barrier. Directional movements, and therefore response patterns, were difficult to attain for the 3 day postoperative worms. But by the sixth day, although corkscrew locomotion was still prevalent, enough coordination had returned to establish directional movements. At this time negative phototropism comprised a majority of the responses (table 1).

At 9 days postoperative many of the worms had regained normal locomotor abilities, although corkscrew movements were still predominant in some. Reversal or withdrawal movements, which originate from posterior segmental contractions and were never seen in the 3 and 6 day postoperative worms, had reappeared but were accomplished only with enormous effort and

uncoordination. When the 9 day worms were placed in front of an illuminated barrier, a condition that requires reversal to escape, corkscrew movements returned.

The 12 and 15 day postoperative worms exhibited normal advance and withdrawal movements. Furthermore, the phototropic responses in each of the four illumination conditions were identical to normal worms. It is important to note at this time that light microscopic data showed that functional neurons were first found in the 13 day regenerating suprapharyngeal ganglion (Schwartz, 1932).

A second group of worms underwent surgery, had their suprapharyngeal ganglia removed, and were subsequently tested with a low intensity light source (14 foot candles). Surprisingly, these worms rarely exhibited the corkscrew movements that were prevalent in the early postoperative days of the first group. The majority of responses were negatively phototropic throughout the testing period and normal response patterns had returned by 12 or 15 days (table 2).

Similar to the first group, the 3, 6, and 9 day postoperative worms rarely exhibited reverse locomotion when they were placed against a lighted barrier or when their anterior segments were illuminated. No worm had any difficulty withdrawing when its posterior segments were illuminated but more important, no worm in the 6 day group or later ever entered a beam of light that was placed adjacent to its body.

A cumulative report on the data from these two groups of worms (table 3) showed that although negative phototropic responses were reduced after the suprapharyngeal ganglion was ablated, they were not completely

abolished, nor was there a strong reversal in the pattern of response. Strong avoidance reactions were evident in the 9 day postoperative worm and by 12 or 15 days the responses were normal.

Since there was a difference in the locomotion patterns of the two groups of worms it was thought that surgical trauma might be responsible for the corkscrew movements. This idea was reinforced by the fact that greater skill and decreased operating time were attained by the time the second group underwent surgery. Trauma to the subpharyngeal ganglion was also considered since difficulty in executing coordinated movements had been described after this ganglion is removed (Bullock and Horridge, 1965).

To determine if trauma affected locomotion or the type of phototropic response elicited by suprapharyngeal ganglion ablated earthworms, a sham operation was performed. The procedure was the same as that used for the two previous groups. The worms recovered quickly and results show that they exhibited negative phototropic responses similar to normal 3 days after surgery (table 4). However, one worm from the five that were tested did exhibit corkscrew movements. They were dominant in the 3 day post-operative test, but did not severely handicap directional movement. They were not seen at 6 days.

V. DISCUSSION

The ultrastructural study of neural elements in the normal supra-pharyngeal ganglion supports several theories of earlier investigators. Scharrer and Brown (1961) found that an increased production of neurosecretory products in the perikaryon caused the fragmentation of organized rough endoplasmic reticulum into free ribosomes. Since protein synthesis primarily occurs in the "Nissl substance", fragmentation acts as a negative feedback mechanism that controls the production rate of neurosecretory material. The neuron cell body in figure 16 supports this theory since it contains a large concentration of vesicles and only a small amount of rough endoplasmic reticulum. This cell is in the storage phase of its secretory cycle. The transport of vesicles down the axon and their subsequent release will inhibit the negative feedback, allowing free ribosomes to reorganize and begin a new phase of production.

Several theories concerning the presence of different types of vesicles in a single bouton in the neuropile of higher animals, including vertebrates, may also be applicable to the earthworm. Kobayashi (1961) theorized that small, clear vesicles (300-500Å) are simply the membranes that are produced after neurosecretory vesicles release their products. For the earthworm, however, this theory is refuted by the fact that Rosenbluth (1972) found acetylcholine within these small "synaptic" vesicles.

Gershenfeld (1960) suggested that synaptic vesicles effect the release of neurosecretory products when they are present in the same axon.

They presumably have an intracellular function, changing either the permeability of the neurosecretory granule or of the presynaptic membrane. Since Gershenfeld found "mixed" boutons only in true neurohaemal organs where there is no synapse on an effector cell, the theory has limited practicality for the earthworm. Its neuropile (fig. 18) contains "mixed" boutons that are involved in synapses between sensory cells and interneurons or effectors, and thus the bouton may function differently.

The investigations of Gabe (1954) suggest that neurosecretory neurons may be secretomotor to the invertebrate equivalent of the pituicyte, inducing the glial cell to release its own neurosecretory product. Paraldehyde fuchsinophilic inclusions have been found in invertebrates (Pipa, 1961). Numerous gliosomes were found throughout the cells in the suprapharyngeal ganglion in this investigation. Several large, osmiophilic particles are shown in figure 17, but they are located in a vascular lacunae and their source and function are unknown.

The light and electron microscopic data of this investigation support the earlier findings of Schwartz (1932) concerning the stem cells that form functional neurons, the nuclear and cytoplasmic transformations that accompany their metamorphosis, and the amount of time required for regeneration of an excised suprapharyngeal ganglion. The infiltration of the ablated area by extracellular vesicles containing catecholamines in the 3 day post-operative tissue (figs. 3,4) suggests a stimulus for the onset of regeneration. Schwartz did not observe these vesicles and suggested instead that trauma to the muscle ventral to the ablated ganglion served as the "exciting agent". Furthermore, Schwartz claimed that the nerve cell bodies

of the subpharyngeal ganglion remained unchanged after the operation. Figure 5 clearly shows an accumulation of catecholamine within their perikaryon. The number of cells that contained this material (approximately 5) was greater than the one or two cells that Schmid found in the normal suprapharyngeal ganglion. These findings support the hypothesis that the vesicles originate in the subpharyngeal ganglion and act as the stimulus for the transformation of connective tissue into nerve in the region of the ablated suprapharyngeal ganglion. This does not preclude the possibility that other ganglion cells throughout the CNS may also produce the same "activating" neurosecretory molecule. Furthermore, the reports that neurosecretory products of the suprapharyngeal ganglion of Lumbricus are required for a 2-3 day period to initiate regeneration of amputated caudal segments (Hubl, 1953, 1956) lend support to the hypothesis that similar neurosecretory products are also required for the regeneration of amputated anterior segments (Goldfarb, 1909) or the regeneration of an ablated suprapharyngeal ganglion.

This investigation also found three different cell types that are representative of the stages of connective tissue metamorphosis to nerve. They are: (1) fibroblast-like cells; (2) "transitional" cells; and (3) neurons. The "fibroblasts" originate dorsolateral to the cut circumpharyngeal connectives, infiltrate the ablated area, and form the stem cells of the new ganglion, dividing and undergoing nuclear and cytoplasmic transformation. The first sign of metamorphosis is the simultaneous decrease in the concentration of nuclear chromatin and increase in the cytoplasmic concentration. This inverse relationship was also described by Schwartz. The

external morphology of the cells also changes as both the nucleus and cytoplasm begin to "round up" from their original elongated form.

The first transitional cells are found in the 9 day postoperative tissue. The characteristic elongation of the cytoplasm and the slightly eccentric nucleus distinguishes them from true nerves. Schwartz (1932) also noted that cells of this stage are incapable of dividing. Apparently they have lost the plasticity of the original stem cells and this had important implications. It is evident that if the transitional cells cannot divide neither can the nerve cells which they will soon form. Since nerve cells cannot divide, then those within the circumpharyngeal connectives, the large pharyngeal nerves, and the nerves innervating the body musculature cannot be a source of new neurons in the regenerated ganglion. This means that the connective tissue cells are the only stem cells of regenerated neurons and therefore, mesodermal cells can assume ectodermal function under suitable conditions. It is still possible, however, that these "connective tissue" stem cells represent a form of tissue that is less differentiated than the supportive connective tissue in other regions of the body. They could then act as stem cells for organs of ectodermal, mesodermal, or endodermal origin. This would explain how these same stem cells form the glia (an epidermal derivative according to Bullock and Horridge) within the regenerated suprapharyngeal ganglion.

The newly formed ganglion cell must synthesize the various products required to function normally. This includes neurohormones or neurotransmitters, various enzymes, axoplasmic components, and the subunits of various organelles that were scanty or absent in the 26 day neurons. Such

synthesis requires ribosomes and this may explain the large concentration of cytoplasmic chromatin in the 26 day cell. The question must be raised, however, as to whether the RNP of the cell, primarily in the rosette configuration or as free ribosomes, is the functional unit of biochemical translation. Scharrer and Brown described the organized rough endoplasmic reticulum as the source of neurohormones. Therefore the RNP of the 26 day cell may represent a nonfunctional stage in the formation of organized Nissl substance.

The 34 day ganglion cell represents the second stage of cytoplasmic transformation, and does not support the idea that free ribosomes are non-functional. The cytoplasmic chromatin has apparently returned to normal levels and has been replaced by an elaborate matrix of agranular endoplasmic reticulum (Golgi apparatus). It is important to note that the ribosomes of the 34 day cell have not formed the Nissl substance characteristic of normal neurons. Although RNP particles are aligned along the membranes of irregular cisterns, the cytoplasm still contains many free ribosomes. It is therefore possible that they have formed cellular products that stimulate the second stage of transformation. This stage is characterized by the packaging of material as shown by the fragmentation of the peripheral borders of the Golgi apparatus and the formation of membrane bound vesicles which accumulate in the cytoplasm.

It is unusual that the processes of synthesis and packaging dominate separate and specific periods of time in the developing ganglion cell. This is not characteristic of normal nerves in which both processes occur simultaneously. It is possible that the dramatic changes that must take

place in the 13 day metamorphosis of "connective tissue" to nerve selectively limit the cytoplasm to one biochemical process at a time, and that completion of one (synthesis) initiates the onset of the next (packaging).

The phototropic response studies of this investigation conflict with earlier reports and show that negative phototropism is not completely abolished following excision of the suprapharyngeal ganglion. It is therefore likely that the ganglion, although an important integrator of sensory information, is not an absolute necessity for light avoidance responses. Although some degree of the normal avoidance behavior is interrupted by the ablation, normal responses return between 9 and 12 days postoperative, well before the suprapharyngeal ganglion has regenerated significantly or formed functional neurons.

The studies of Hess showed that the suprapharyngeal ganglion ablated earthworms were positively phototropic until 14 to 24 days postoperative, when avoidance responses again became predominant. At 32 days the worms exhibited normal light avoidance behavior. Hess' findings (8.3% avoidance responses at one day postoperative and a 52.1% avoidance response at 16 days) are interesting when compared with the histological features of regeneration. Negative phototropism became predominant only 3 days after the first functional neurons were formed; only one day after the fibers of the regenerating suprapharyngeal ganglion had decussated and rejoined the circumpharyngeal connectives; and 5 days before the prostomial nerve trunks had rejoined the ganglion. Although these responses can be attributed to some degree of regeneration, it is unlikely that a large group of new cells would have

simultaneously formed the functional synapses between themselves, the fibers of the connectives, and the afferent fibers from the prostomium or the ventral nerve cord necessary to integrate sensory information to any great extent. Such an event would have had to happen within 3 days after the first true neurons were formed. Although the 18 day postoperative tissue revealed many normal nerve cell bodies, the neuropile did not have the extensive synaptic regions characteristic of normal tissue. Furthermore, Hess noted a gradual shift in negative phototropism from 8.3% one day postoperative to 23% at 12 days - one day before any neurons had even been formed. The sham operation conducted in this investigation showed that surgical trauma had no effect on light avoidance behavior (84% negative at 3 days postoperative), but was possibly responsible for the uncoordinated movements found in ganglion ablated worms. It is therefore unlikely that the change in response found in the 22 day postoperative worms of Hess' experiment was a result of recovery from surgical trauma, but it is possible that the change can be attributed to the procedure used to determine responses. Therefore the testing procedure must be examined to determine whether negative phototropic responses were recorded for the correct behavior.

The significant variable in these experiments was the illumination period, which was extended from 30 seconds to 70 seconds/trial in the postoperative tests. This was done for several reasons. Early postoperative worms experienced difficulty in establishing coordinated movements. Therefore the response time was longer and if the illumination period had not been increased the responses would have become dependent on two new

variables: (1) the general health of the worms and their ability to regain normal locomotion; (2) the diameter of the beam of light. If the worms were unhealthy and had difficulty moving, a short illumination period would have caught them in the beam of light and been recorded as a positive response, regardless of whether they were attempting to escape. The time extension seemed appropriate since it was found that normal worms would avoid light regardless of how long they were illuminated. If suprapharyngeal ganglion ablated worms were positively phototropic, they should remain in the light regardless of the length of the testing period. Similarly, an unhealthy worm, if negatively phototropic, should avoid the light regardless of how long it took to escape.

Another important factor are the alternative anatomical pathways for reflex arcs that involve photoreceptors. Hess (1925) was the first investigator to describe the subepidermal nerve plexus in detail. The sensory receptors of the epithelium, including photoreceptors, have two fiber projections. One passes by way of the segmental nerve to the CNS, and the second joins the plexus and synapses on interneurons or directly on the longitudinal and circular body musculature, forming a one or two cell reflex arc. The plexus is also non-segmental, permitting responses to extend over several segments from the stimulated area. Hess found that efferent fibers from the CNS unite with those of the plexus in the body musculature. He theorized that the plexus controlled local contractile responses that were positively phototropic unless they were inhibited by fibers from the CNS, that were, in turn, controlled by the suprapharyngeal ganglion.

In view of the results of this investigation, it is assumed that the subepidermal plexus may be responsible for adaptive movements that are more complicated than a positive phototropic reflex would permit. Hess' theory does not consider the role of the plexus in translating other receptor stimuli (chemical and tactile) into specific movements. On the whole, however, it seems questionable that the entire plexus simply acts as an antagonist to the CNS. Since the earthworms represents an evolutionary transition from the plexus to the central nervous system, it may be possible that the subepidermal plexus can produce the same response patterns as the CNS, but on a simpler scale. Therefore, although the suprapharyngeal ganglion is the important phototropic sensory integrator in the normal earthworm, the same function could be accomplished by the subepidermal plexus in its absence. This would account for the negative phototropic responses seen in suprapharyngeal ganglion ablated worms.

VI. CONCLUSION

The results of this investigation substantiate the findings of Schwartz concerning the histological features of regeneration of neural tissue following the excision of the suprapharyngeal ganglion. Cells which are morphologically similar to connective tissue infiltrate the ablated area and are transformed into nerve within 13 days. The new ganglion rejoins the circumpharyngeal connectives and prostomial nerves by 21 days. Three stages of transformation are distinguished by the cytoplasmic, nuclear, and morphological changes of the metamorphosing cells. They include: (1) A "fibroblast" stage approximately five days in length. The cells present an eccentric nucleus containing homogeneous regions of chromatin, and an elongated cytoplasm devoid of Nissl substance. (2) A "transitional" stage from the sixth to twelfth days, characterized by a simultaneous decrease in nuclear and an increase in cytoplasmic material. The cells become rounded, more characteristic of nerve than connective tissue. Late in this stage a cytoplasmic evagination demarcates the location of the developing axon. (3) A final neural stage, characterized by functional ganglion cells and a well developed neuropile. After a relatively "normal" period, the perikaryon undergoes a change that lasts approximately four days, in which the cytoplasm exhibits an unusually large concentration of Nissl substance.

A possible "activating" molecule has been observed in the early post-operative tissue that may control the metamorphosis of connective tissue to nerve. The molecule was found within extracellular vesicles in the ablated area and revealed a chromaffin reaction after potassium dichromate fixation.

The subpharyngeal ganglion was identified as one possible source of the molecule since several of its cells revealed the same chromaffin reaction.

This investigation is the first to describe the ultrastructure of the cells found within the regenerating suprapharyngeal ganglion. The 12 day postoperative tissue is primarily filled with invading connective tissue and muscle from areas ventral to the ablation. The 18 day ganglion displays many nerve cells and a well developed neuropile. Following this relatively normal phase, the neurons undergo two stages of cytoplasmic transformation. The first is characterized by the 26 day ganglion, whose cells are filled with many RNP particles, primarily as free ribosomes. This is assumed to be correlated with intense protein synthesis. The second stage is found in 34 day ganglion cells whose cytoplasm contains an elaborate matrix of agranular endoplasmic reticulum. This signifies a change from synthesis to packaging of cellular products. The striking feature is that these two stages of metabolic activity, which occur simultaneously in the normal neuron, dominate separate periods in the developing ganglion cell.

The phototropic response studies conducted in this experiment show that suprapharyngeal ganglion ablated earthworms do not exhibit drastic changes from their normal light avoidance behavior. Although negative phototropic responses are reduced for a period of nine days, they return by the twelfth or fifteenth. This is approximately six days before the regenerated ganglion can be considered functional. The ganglion ablated worms never exhibited the strong reversal in response (positive phototropism) that had been described by other investigators. The absence of this reversal may be attributed to two factors. The first is the criteria that was used to

determine response patterns. It was found that the exposure time had to be increased for early postoperative worms in order to verify the correct response. This was because of the difficulty that these worms had in establishing directional movements. It was reasoned that true positive or negative phototropic behavior should be maintained regardless of the exposure time (i.e. exposure time should be an independent variable). By fixing a specific exposure time, earlier investigators made the responses of the ganglion ablated worms dependent on 2 new variables: (1) the diameter of the light beam; (2) the ability of the worms to regain normal locomotion. By eliminating these variables correct responses could then be recorded.

The second important factor are the alternate neuronal pathways that may function as sensory integrators. One logical possibility is the subepidermal nerve plexus. Instead of the antagonistic role described by Hess (1925), this plexus may operate in conjunction with the CNS in determining phototropic responses. The removal of the suprapharyngeal ganglion still leaves the subepidermal plexus to maintain the light avoidance behavior observed in ganglion ablated earthworms.

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TABLE 1

Reactions to a Moderate Light Intensity (56 foot candles) for
Earthworms on Successive Days After Ganglion Removal

| Day | Worm | Advance | Retreat | No Response | Total Responses | Percent Advance | Percent Retreat |
|--------|-------|---------------|----------------|----------------|--------------------|--------------------|--------------------|
| Normal | 30-48 | 31 (17.3%) | 133 (74.3%) | 15 (8.4%) | 164 | <u>18.9%</u> | <u>81.1%</u> |
| 3 | 1,2 | 13 | 6 | 1 | 19 | 68.4 | 31.6 |
| | 5-7 | 8 | 11 | 7 | 19 | 42.1 | 57.9 |
| | Total | 21 (45.7%) | 17 (37.0%) | 8 (17.3%) | 38 | <u>55.3%</u> | <u>44.7%</u> |
| 6 | 8-11 | 32 (57.1%) | 23 (41.1%) | 1 (1.8%) | 55 | <u>41.8%</u> | <u>58.2%</u> |
| 9 | 5,6 | 3 | 16 | 1 | 19 | 15.8 | 84.2 |
| | 8-13 | 13 | 40 | 4 | 53 | 24.5 | 75.5 |
| | 14-17 | 15 | 20 | 4 | 35 | 42.9 | 57.1 |
| | 18-21 | 10 | 28 | 2 | 38 | 26.3 | 73.7 |
| | Total | 41 (26.3%) | 104 (66.6%) | 11 (7.1%) | 145 | <u>28.3%</u> | <u>71.7%</u> |
| 12 | 8-12 | 6 (14.0%) | 34 (79.1) | 3 (6.9%) | 40 | <u>15.0%</u> | <u>85.0%</u> |
| 15 | 1-4 | 7 | 26 | 6 | 33 | 21.2 | 78.8 |
| | 14-17 | 9 | 27 | 4 | 36 | 25.0 | 75.0 |
| | Total | 16 (20.3%) | 53 (67.1%) | 10 (12.6%) | 69 | <u>21.6%</u> | <u>78.4%</u> |

TABLE 2

Reactions to a Low Light Intensity (14 foot candles) for
Earthworms on Successive Days After Ganglion Removal

| Day | Worm | Advance | Retreat | No Response | Total Responses | Percent Advance | Percent Retreat |
|-----|-------|---------------|---------------|----------------|--------------------|--------------------|--------------------|
| 3 | 22-25 | 15 | 25 | 0 | 40 | <u>37.5%</u> | <u>62.5%</u> |
| 6 | 26-29 | 10 | 30 | 0 | 40 | <u>25.5%</u> | <u>75.0%</u> |
| 12 | 18-20 | 6 (20.0%) | 23 (76.7%) | 1 (3.3%) | 29 | <u>20.7%</u> | <u>79.3%</u> |
| 15 | 8-11 | 6 (15.0%) | 32 (80.0%) | 2 (5.0%) | 38 | <u>15.8%</u> | <u>84.2%</u> |
| 18 | 8-11 | 4 | 35 | 1 | 39 | 10.3 | 89.7 |
| | 26-28 | 7 | 21 | 2 | 28 | 25.0 | 75.0 |
| | Total | 11 (15.7%) | 56 (80.0%) | 3 (4.3%) | 67 | <u>16.4%</u> | <u>83.6%</u> |
| 21 | 1-3 | 5 | 24 | 1 | 29 | 17.2 | 82.8 |
| | 8-11 | 6 | 34 | 0 | 40 | 15.0 | 85.0 |
| | 14-17 | 6 | 34 | 0 | 40 | 15.0 | 85.0 |
| | Total | 17 (15.5%) | 92 (83.6%) | 1 (0.9%) | 109 | <u>15.9%</u> | <u>84.1%</u> |
| 27 | 1-4 | 8 | 29 | 3 | 37 | 21.6 | 78.4 |
| | 8,9 | 3 | 17 | 0 | 20 | 15.0 | 85.0 |
| | 14-17 | 6 | 33 | 1 | 39 | 15.4 | 84.6 |
| | Total | 17 (17.0%) | 79 (79.0%) | 4 (4.0%) | 96 | <u>17.7%</u> | <u>82.3%</u> |

TABLE 3

Cumulative Reactions to Light Stimuli for Earthworms on
Successive Days After Ganglion Removal

| Day | Advance | Retreat | No Response | Total Responses | Percent Advance | Percent Retreat |
|--------|---------|---------|----------------|--------------------|--------------------|--------------------|
| Normal | 31 | 133 | 15 | 164 | 18.9% | 81.1% |
| 3 | 36 | 42 | 8 | 78 | 46.2% | 53.8% |
| 6 | 33 | 62 | 1 | 95 | 34.7% | 65.3% |
| 9 | 41 | 104 | 11 | 145 | 28.3% | 71.7% |
| 12 | 12 | 57 | 4 | 69 | 17.4% | 82.6% |
| 15 | 22 | 85 | 12 | 107 | 20.6% | 79.4% |
| 18 | 11 | 56 | 3 | 67 | 16.4% | 83.6% |
| 21 | 17 | 92 | 1 | 109 | 15.9% | 84.1% |
| 27 | 17 | 79 | 4 | 96 | 17.7% | 82.3% |

TABLE 4

Reactions to a Low Light Intensity (14 foot candles) for
Earthworms on Successive Days After a Sham Operation

| Day | Worm | Advance | Retreat | No Response | Total Responses | Percent Advance | Percent Retreat |
|-----|-------|---------|---------|----------------|--------------------|--------------------|--------------------|
| 3 | 49,50 | 1 | 19 | 0 | 20 | 5.0 | 95.0 |
| | 51-53 | 7 | 23 | 0 | 30 | 23.3 | 76.7 |
| | Total | 8 | 42 | 0 | 50 | | |
| | | (16.0%) | (84.0%) | | | <u>16.0%</u> | <u>84.0%</u> |
| 6 | 49,50 | 4 | 16 | 0 | 20 | 20.0 | 80.0 |
| | 51-53 | 4 | 26 | 0 | 30 | 13.3 | 86.7 |
| | Total | 8 | 42 | 0 | 50 | | |
| | | (16.0%) | (84.0%) | | | <u>16.0%</u> | <u>84.0%</u> |

PLATE 1

EXPLANATION OF FIGURES

- Figure 1: Diagram of the CNS of the earthworm taken from Hess (1924). This shows the suprapharyngeal ganglion, the large prostomial nerves originating from its lateral aspect, and its connection to the subpharyngeal ganglion via the circumpharyngeal connectives.
- Figure 2: Normal suprapharyngeal ganglion showing the nerve cell bodies located in its dorsal and lateral aspect surrounding the central neuropile. A portion of the ventral muscle located between the ganglion and the gut is also shown. X52 (thionin and eosin B)

NP = Neuropile

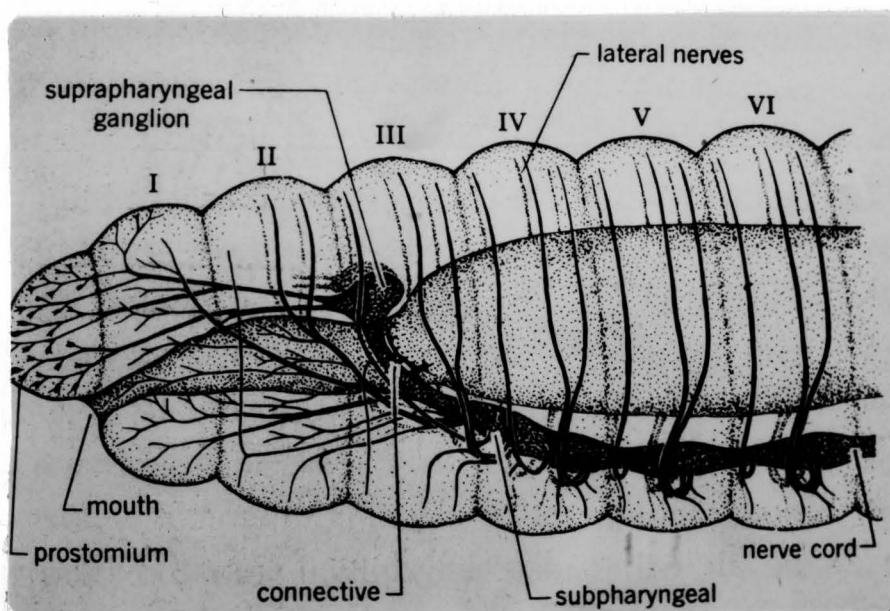


FIGURE 1

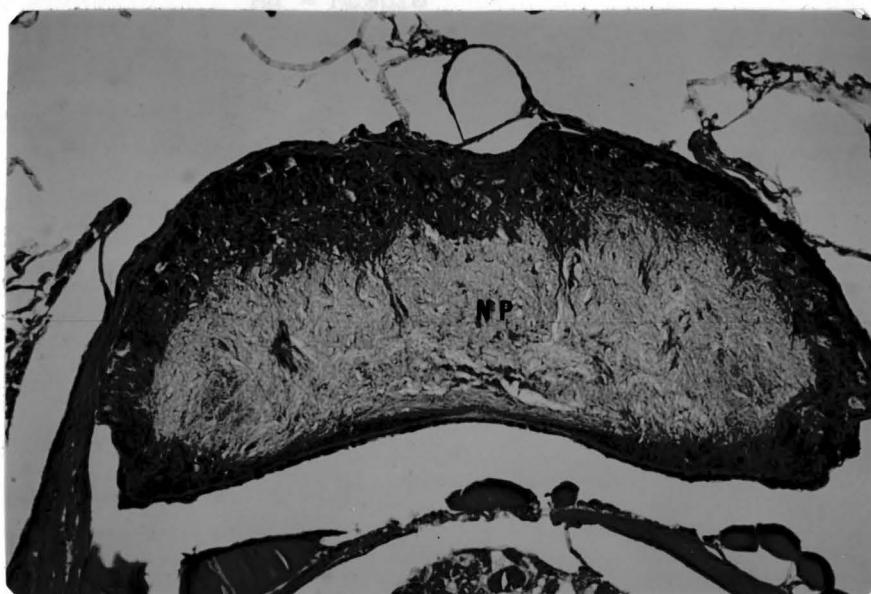


FIGURE 2

PLATE 2

EXPLANATION OF FIGURES

- Figure 3: Numerous extracellular vesicles, muscle and connective tissue, and vessels in the region of the ablated suprapharyngeal ganglion 3 days postoperative. X57 (thionin and eosin B)
- Figure 4: Vesicles within the region of the ablated suprapharyngeal ganglion 3 days postoperative, showing a distinct chromaffin reaction. X334 (thionin and eosin B)

BV = Blood vessels
Mu = Muscle
Ves = Vesicles

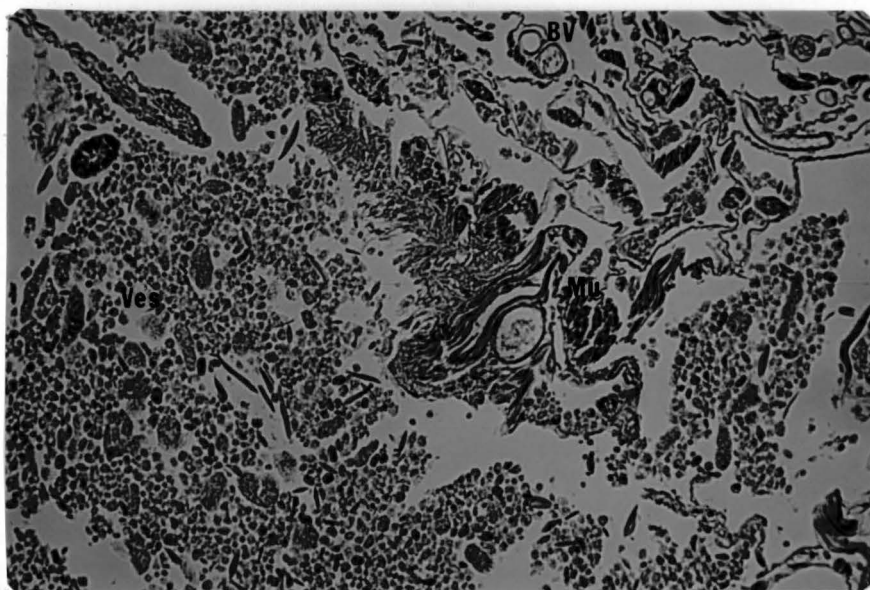


FIGURE 3

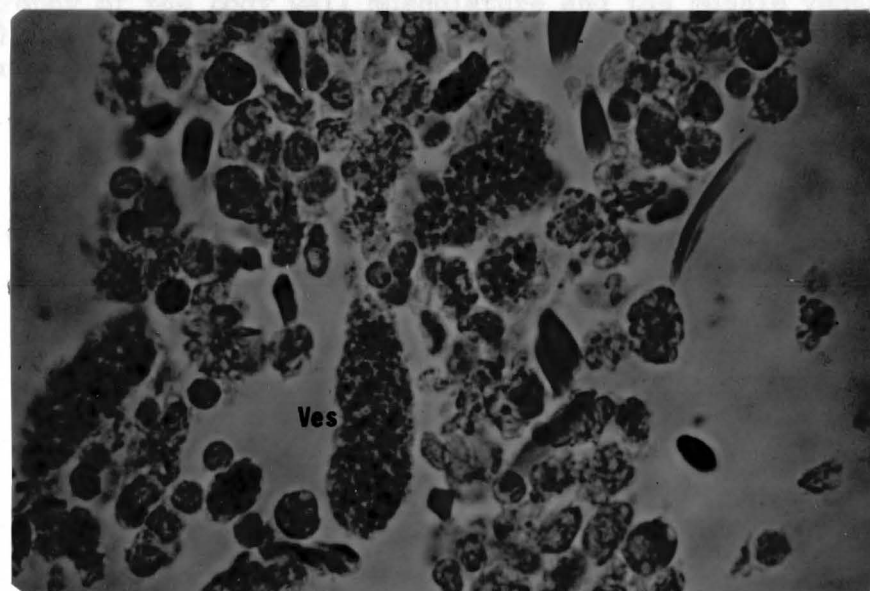


FIGURE 4

PLATE 3

EXPLANATION OF FIGURES

- Figure 5: Ganglion cells within the subpharyngeal ganglion 3 days postoperative. Notice the chromaffin reaction and vacuolization throughout the cytoplasm. Other cells within the field give no indication of secretory activity. X334 (thionin and eosin B)
- Figure 6: Region of the ablated suprapharyngeal ganglion 9 days postoperative showing the "dense" connective tissue bridge extending between the two cut connectives. Note the demuded area above this connective tissue band and the dorsomedial growth of the circumpharyngeal connectives; also the hypertrophy of the body wall musculature and the muscle ventral to the ablated ganglion whose fibers have infiltrated the dense connective tissue band. X52 (thionin and eosin B)

CC = Circumpharyngeal connectives
DCT = Dense connective tissue

Mu = Muscle
Ves = Vesicles

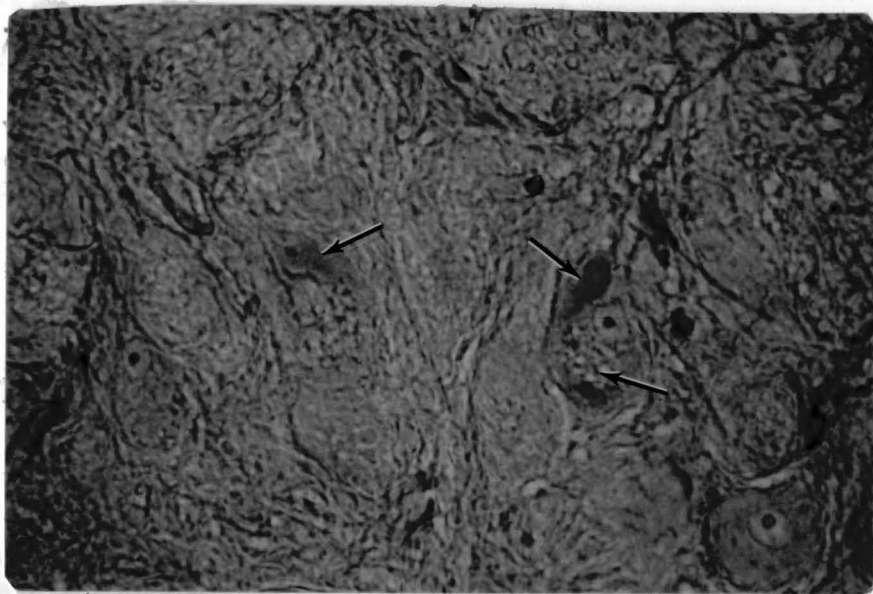


FIGURE 5



FIGURE 6

PLATE 4

EXPLANATION OF FIGURES

Figure 7: Muscle infiltration into the region of the ablated ganglion 12 days postoperative. A portion of the developing nerve cord sheath that encompasses the muscle and connects it to the circumpharyngeal connectives is also shown (arrows). X67 (thionin and eosin B)

Figure 8: Regenerating suprapharyngeal ganglion 15 days postoperative. The ganglion is bilobed and has rejoined the circumpharyngeal connectives. Groups of ganglion cells are found dorsolateral and ventral to the neuropile. Notice the large area of connective tissue surrounding the ganglion. X67 (thionin and eosin B)

CC = Circumpharyngeal connectives
DCT = Dense connective tissue

G = Gut
NP = Neuropile

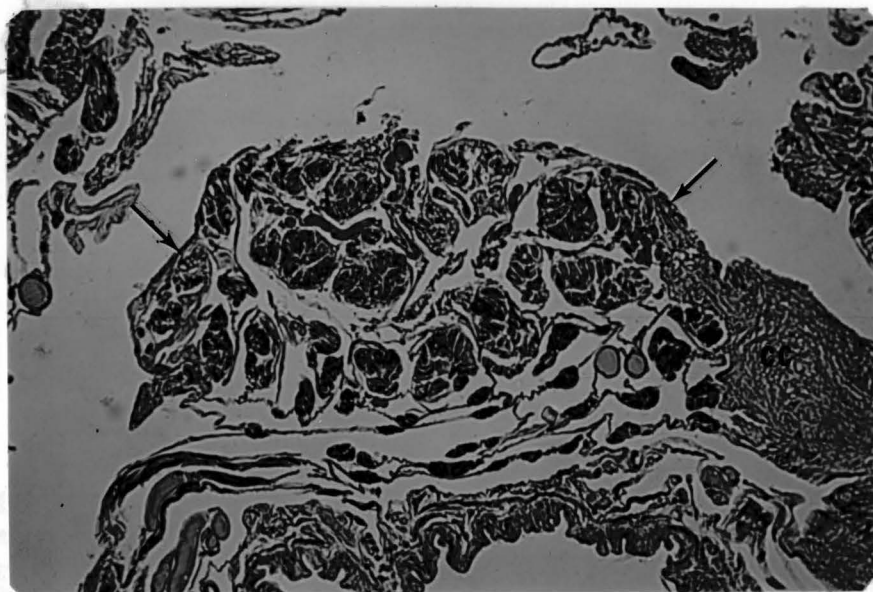


FIGURE 7

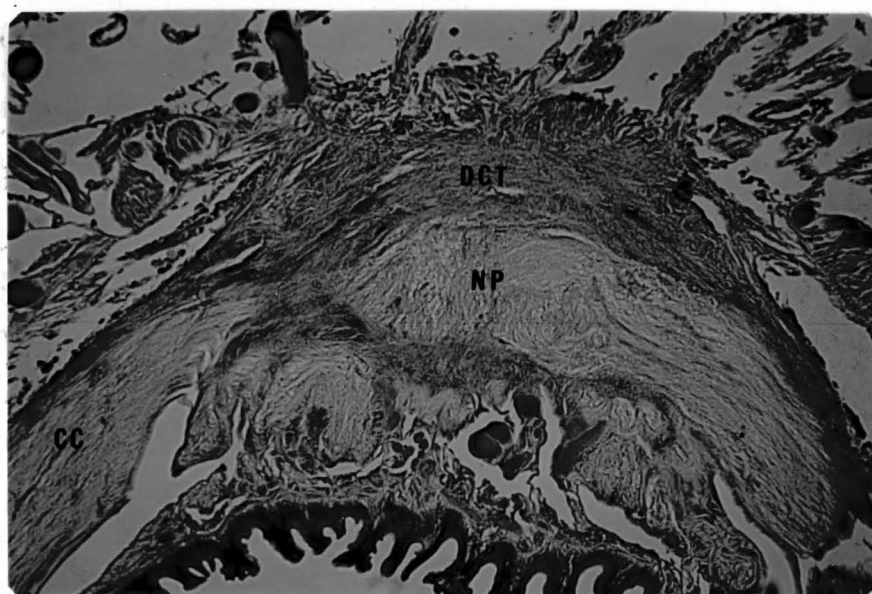


FIGURE 8

PLATE 5

EXPLANATION OF FIGURES

- Figure 9: Fibroblasts in the connective tissue dorsal to the regenerating ganglion in the 15 day postoperative tissue. Notice the eccentric nuclei with homogenous regions of chromatin and the elongated eosin-stained cytoplasm. X836 (thionin and eosin B)
- Figure 10: Transitional cells located ventral to the fibroblasts and dorsal to the nerve cell bodies in the 15 day regenerating ganglion. Notice the elongated nucleus and prominent nucleolus, and the increase in thionin-stained granular endoplasmic reticulum in the cytoplasm. X1672 (thionin and eosin B)

GER = Granular endoplasmic reticulum
N = Nucleus

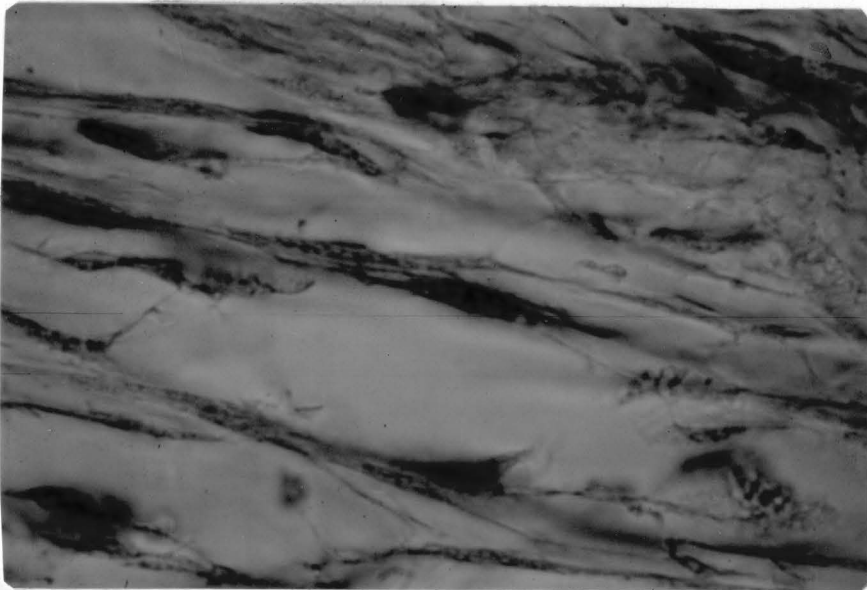


Figure 11: A section of the 10 day regenerating ganglion. The nucleus is now rounded and sparse in chromatin except for a prominent nucleolus. The cytoplasm is decreased. **FIGURE 9** and contains clumps of granular endoplasmic reticulum. X1320 (thionin and eosin B)

Figure 12: Ganglion cells in the 31 day postoperative lesion. Notice the densely stained cytoplasm that appears to be completely filled with granular endoplasmic reticulum. X143 (thionin and eosin B)

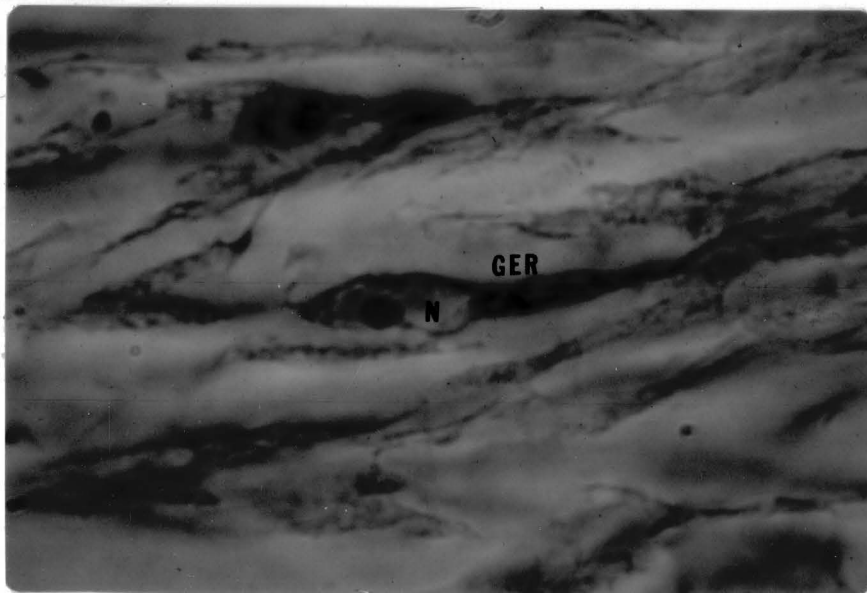


FIGURE 10

PLATE 6

EXPLANATION OF FIGURES

Figure 11: A well developed nerve cell located in the ventral portion of the 15 day regenerating ganglion. The nucleus is now rounded and sparse in chromatin except for a prominent nucleolus. The cytoplasm is decreased in volume and contains clumps of granular endoplasmic reticulum. X1320 (thionin and eosin B)

Figure 12: Ganglion cells in the 30 day postoperative tissue. Notice the densely stained cytoplasm that appears to be completely filled with granular endoplasmic reticulum. X1045 (thionin and eosin B)

GER = Granular endoplasmic reticulum

Nu = Nucleolus

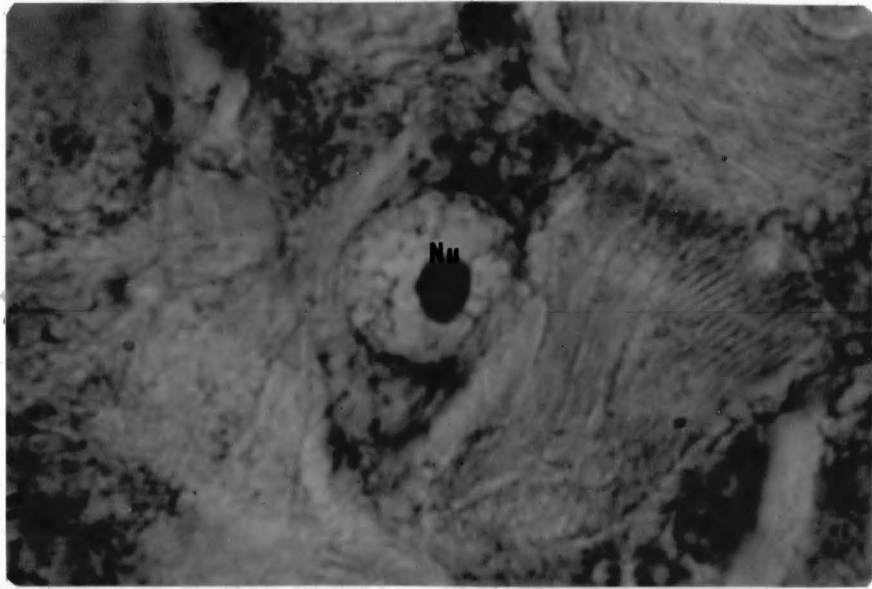


FIGURE 11

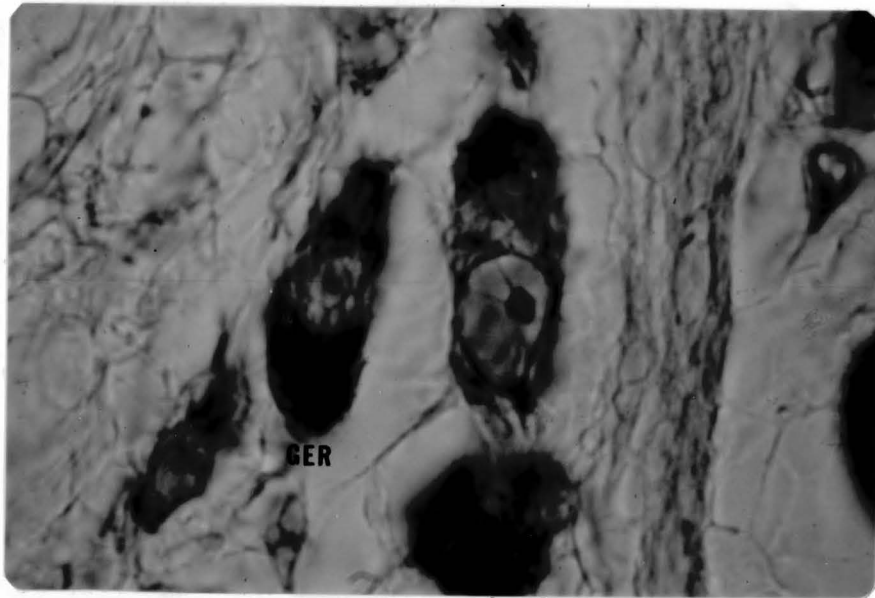


FIGURE 12

PLATE 7

EXPLANATION OF FIGURES

- Figure 13: Eighty-four day suprapharyngeal ganglion as it joins the circumpharyngeal connectives. The histological features of the ganglion appear normal. X67 (thionin and eosin B)
- Figure 14: Nerve cell bodies in the 84 day suprapharyngeal ganglion showing conspicuous areas of thionin-stained granular endoplasmic reticulum throughout the cytoplasm. X1045 (thionin and eosin B)

NP = Neuropile

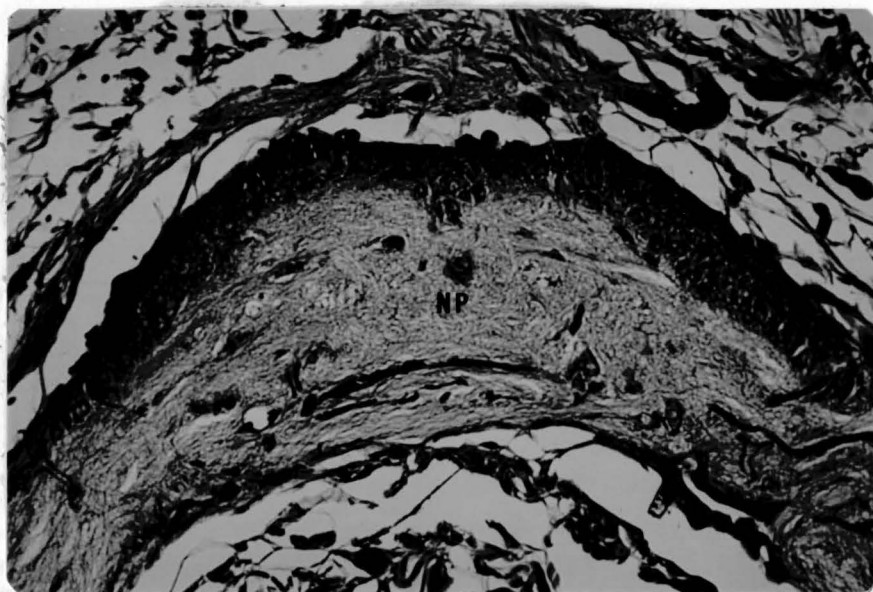


FIGURE 13

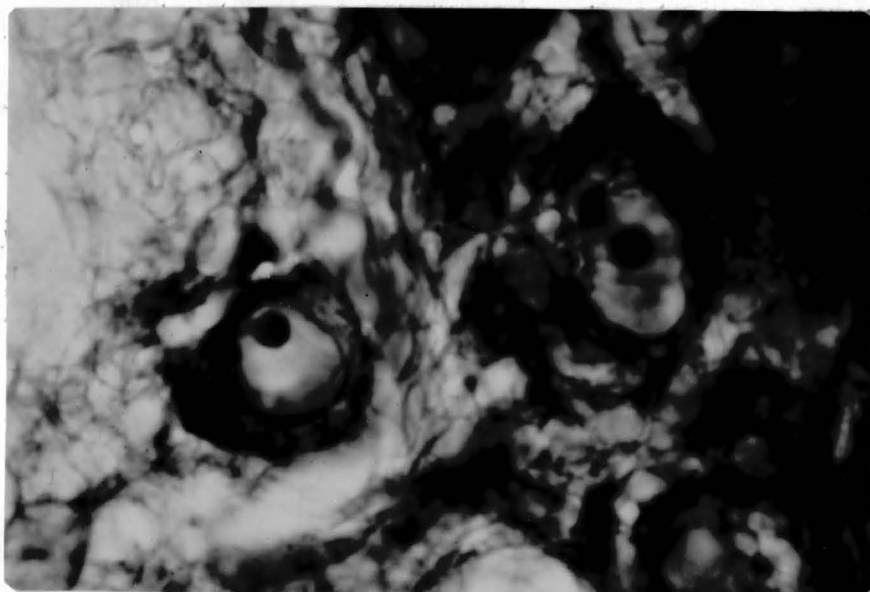


FIGURE 14

PLATE 8

EXPLANATION OF FIGURE

Figure 15: Scanning electron micrograph of the CNS of the earthworm. Notice the exposed suprapharyngeal ganglion that is encompassed in the thick nerve cord sheath. The circumpharyngeal connectives extending to the subpharyngeal ganglion are also shown. Large segmental nerve trunks extend from the ventral nerve cord. X100

CC = Circumpharyngeal connectives
SN = Segmental nerve
Sup G = Suprapharyngeal ganglion
VNC = Ventral nerve cord



FIGURE 15

PLATE 9

EXPLANATION OF FIGURE

Figure 16: Electron micrograph of a normal mixed neurosecretory cell with two populations of cytoplasmic vesicles. Sparse amounts of rough endoplasmic reticulum and numerous free ribosomes; also notice the two cytoplasmic indentations (trophospongium) formed by glial processes (arrows). The neurosecretory vesicles range in diameter from 1200-2600Å. The multivesicular body is 1.4µ wide. X25,200.

Gl P = Glial process
MVB = Multivesicular body

N = Nucleus
Ves = Vesicles

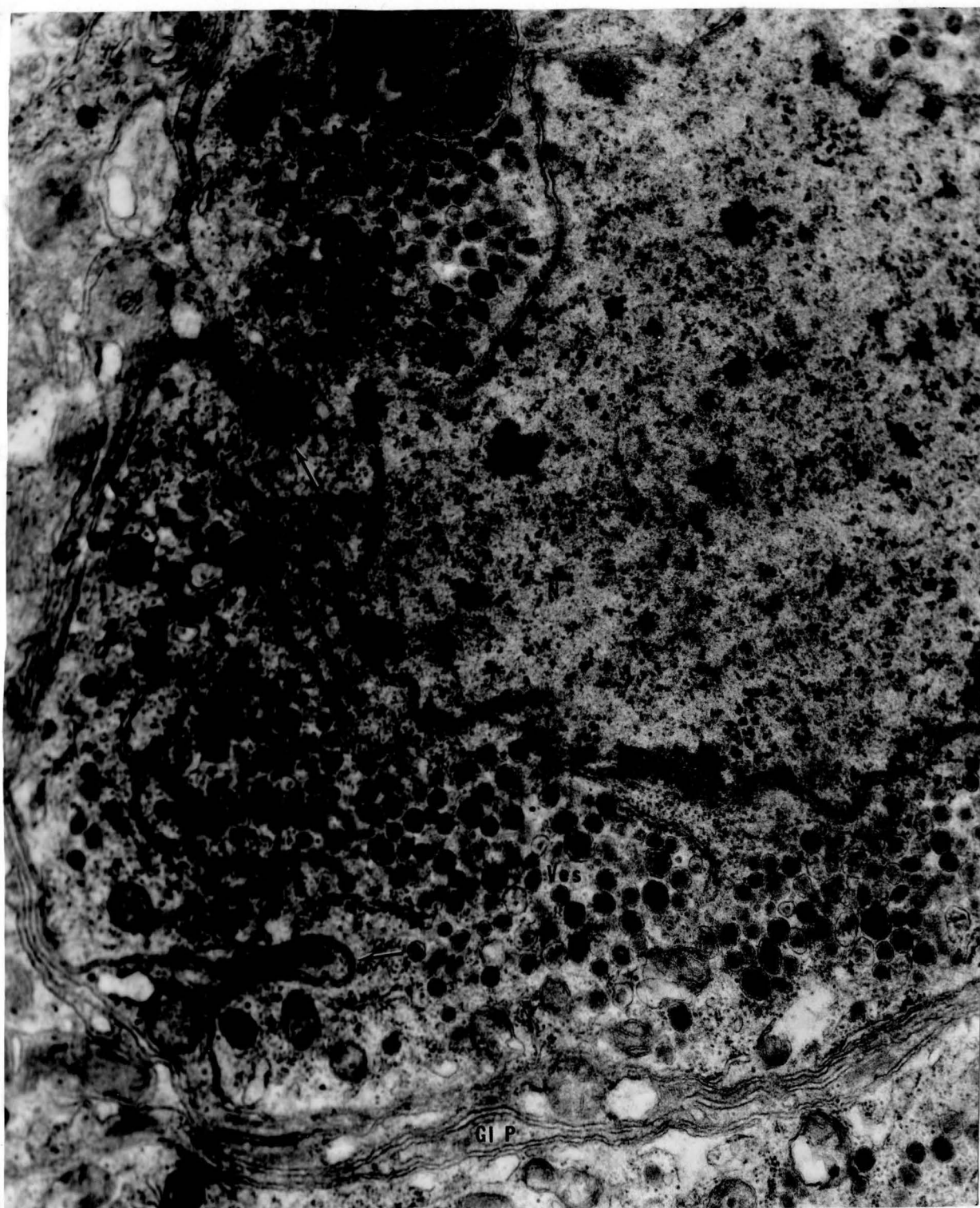


FIGURE 16

PLATE 10

EXPLANATION OF FIGURE

Figure 17: Glial cell located in the nerve cord sheath of the supra-pharyngeal ganglion. The nucleus contains numerous clumps of chromatin. The glial cytoplasm envelopes a single axon (arrow) and many neurofilaments are found throughout this region. X17,000.

Ax = Axon
N = Nucleus
Nf = Neurofilaments

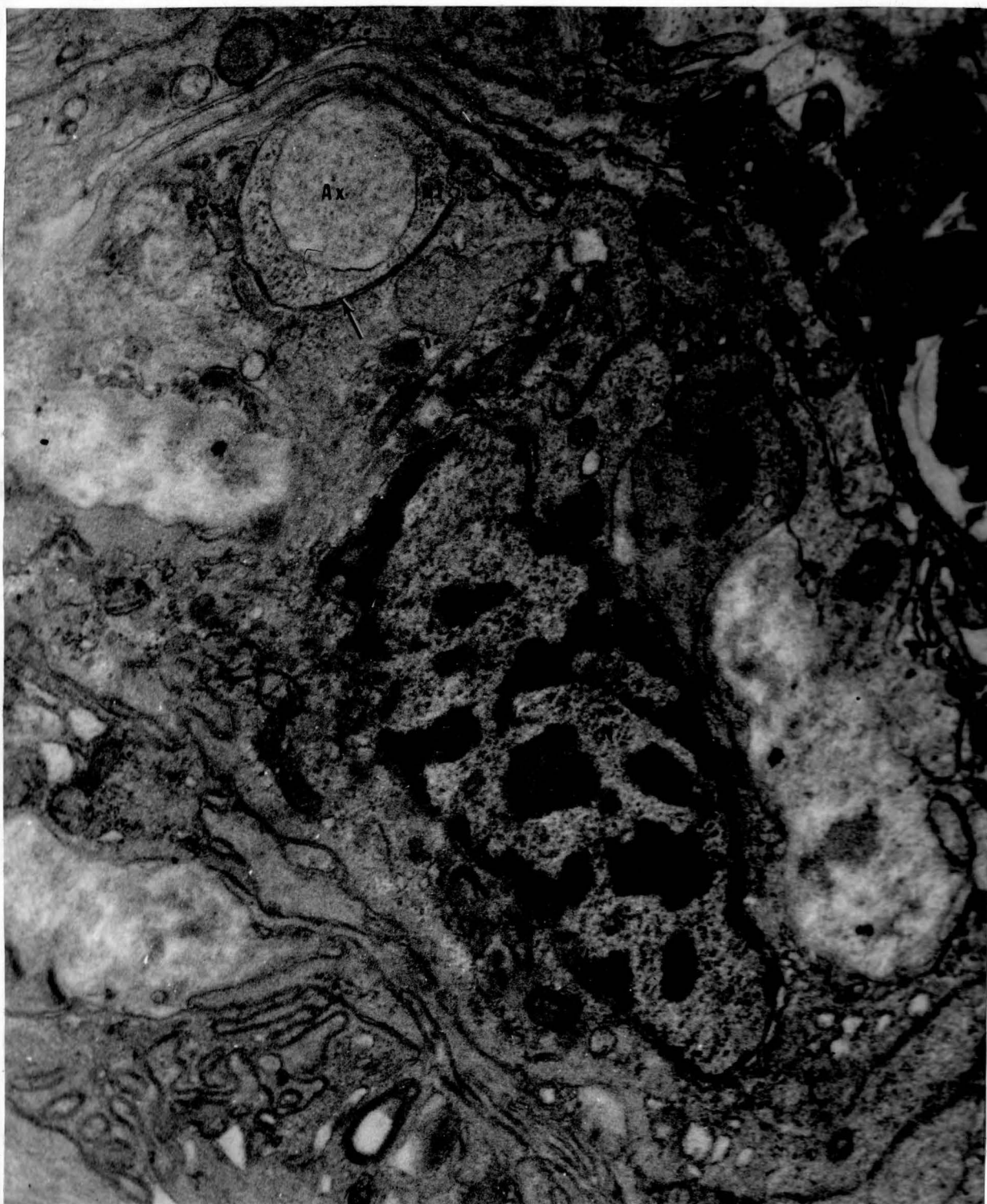


FIGURE 17

PLATE 11

EXPLANATION OF FIGURE

Figure 18: The neuropile of the normal suprapharyngeal ganglion. The axons are filled with many clear "synaptic" vesicles 300-500Å in diameter. A single axon contains both dark halo vesicles (900-1500Å) and clear synaptic vesicles. Notice the absence of any specialized organelles in either the pre-synaptic or postsynaptic bouton. X33,000.

C Ves = Clear vesicles
H Ves = Halo vesicles

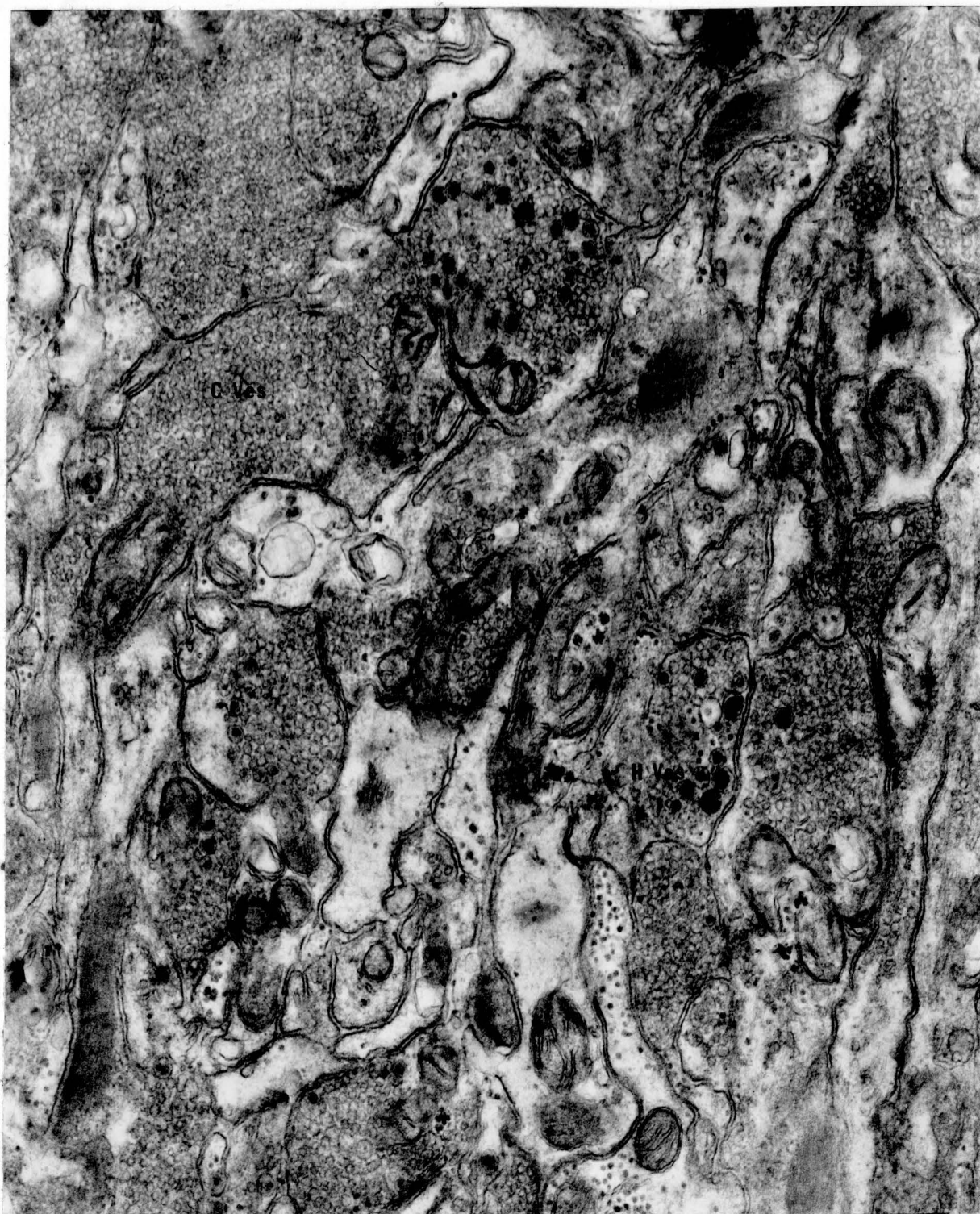


FIGURE 18

PLATE 12

EXPLANATION OF FIGURE

Figure 19: Two muscle bundles in the 12 day postoperative tissue. One bundle is characteristic of normal muscle while the second is undergoing degenerative changes. The diameter of the dense filaments in the second bundle has increased in size, the t-tubules are not readily discernible, and two large myelin figures dominate the field. X49,000.

D = Dense filaments
Mf = Myelin figure
t = t-tubules



FIGURE 19

PLATE 13

EXPLANATION OF FIGURE

Figure 20: A fibroblast nucleus and a portion of its cytoplasm are shown in the 12 day postoperative tissue. The nucleus is elongated but the amount of chromatin is diminishing. The cytoplasm is devoid of organelles other than an extensive system of filaments near the nucleus. X22,400.

F = Filaments
N = Nucleus

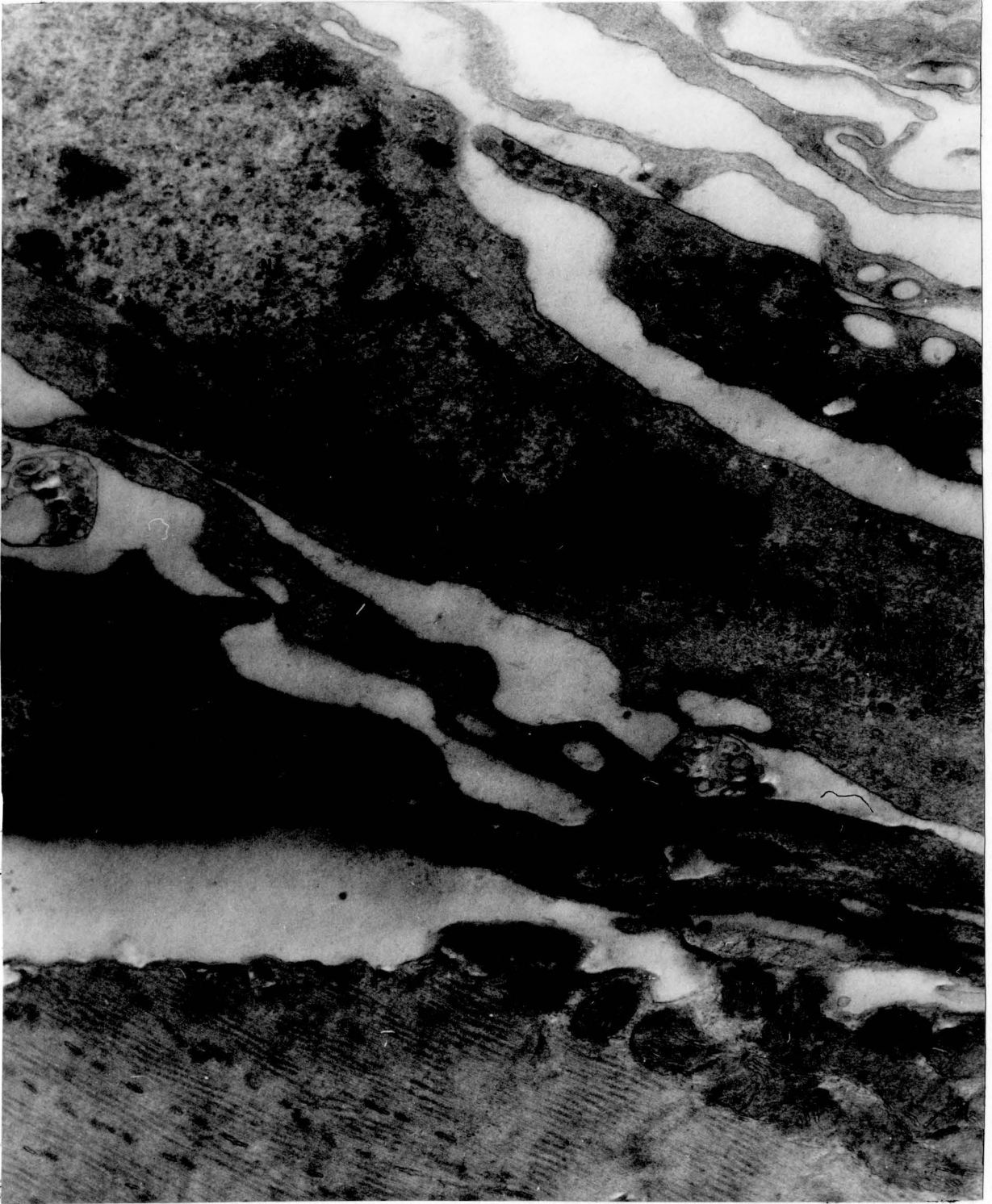


FIGURE 20

PLATE 14

EXPLANATION OF FIGURE

Figure 21: A nerve cell in the 18 day postoperative tissue. The nucleus is round and contains a nucleolus. The cytoplasm contains numerous mitochondria, RNP particles, and an extensive network of filaments surrounding the nucleus. Note also a single matrix of agranular endoplasmic reticulum, some free ribosomes, and a number of elementary vesicles (150Å) nearby. X26,600.

AER = Agranular endoplasmic reticulum
F = Filaments
M = Mitochondrion
N = Nucleus
Nu = Nucleolus
RNP = Ribonucleoprotein (ribosomes)
Ves = Vesicles

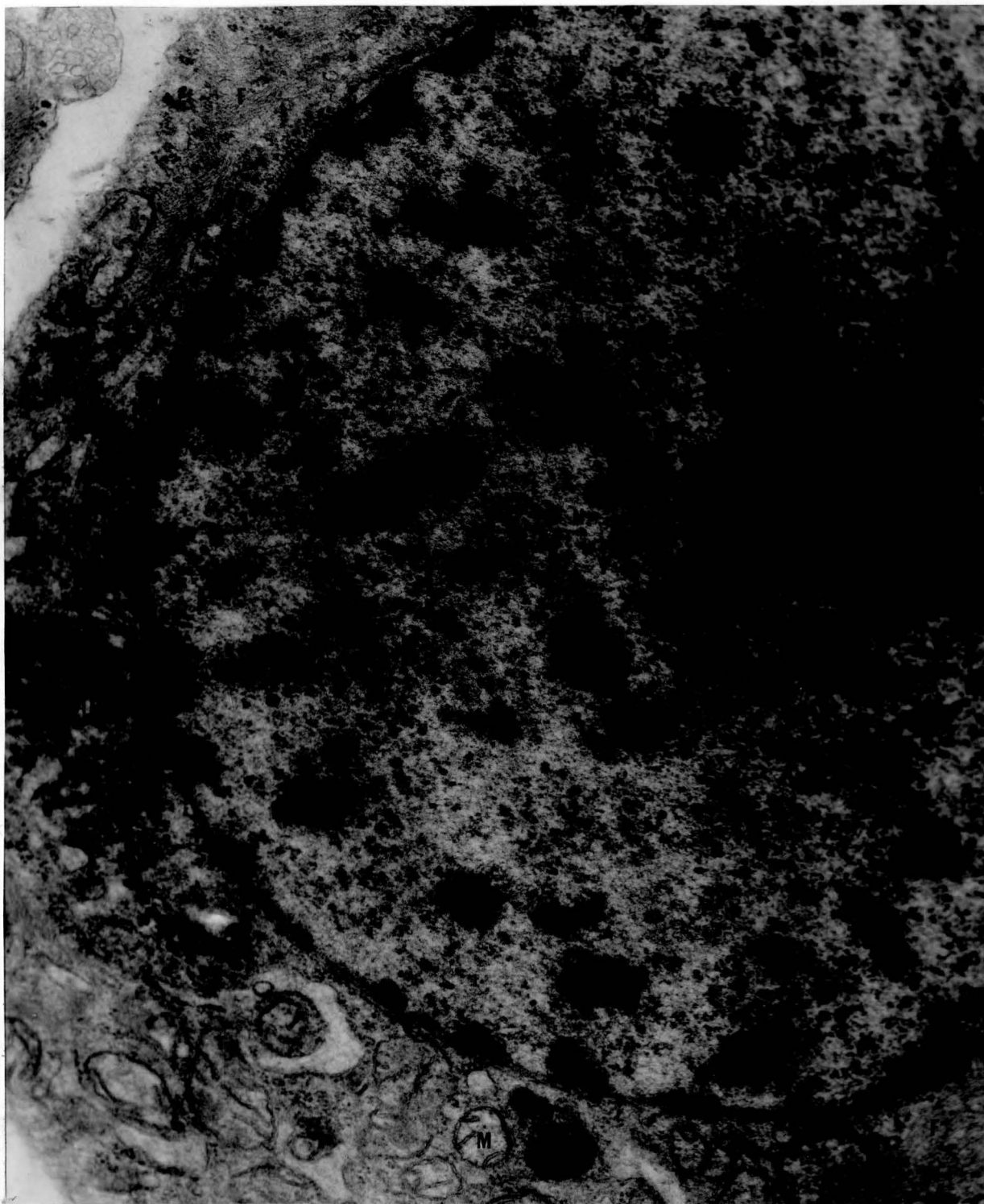


FIGURE 21

PLATE 15

EXPLANATION OF FIGURE

Figure 22: A ganglion cell in the 18 day tissue. The nucleus contains a prominent nucleolus. The cytoplasm is filled with many RNP particles in both the rosette configuration and as free ribosomes. A number of ribosomes are aligned along unusually wide cisterns approximately $.35\mu$ in diameter. X44,000.

Cis = Cisterns

N = Nucleus

Nu = Nucleolus

RNP = Ribonucleoprotein (ribosomes)



FIGURE 22

PLATE 16

EXPLANATION OF FIGURE

Figure 23: Developing neuropile within the 18 day suprapharyngeal ganglion. Vesicles filling the terminal boutons are similar to those in the normal neuropile although there appears to be two different populations of clear "synaptic" vesicles, based on size. One group ranged from 400-500Å while the second contains vesicles 950-1100Å in diameter. X79,750.

C Ves = Clear vesicles

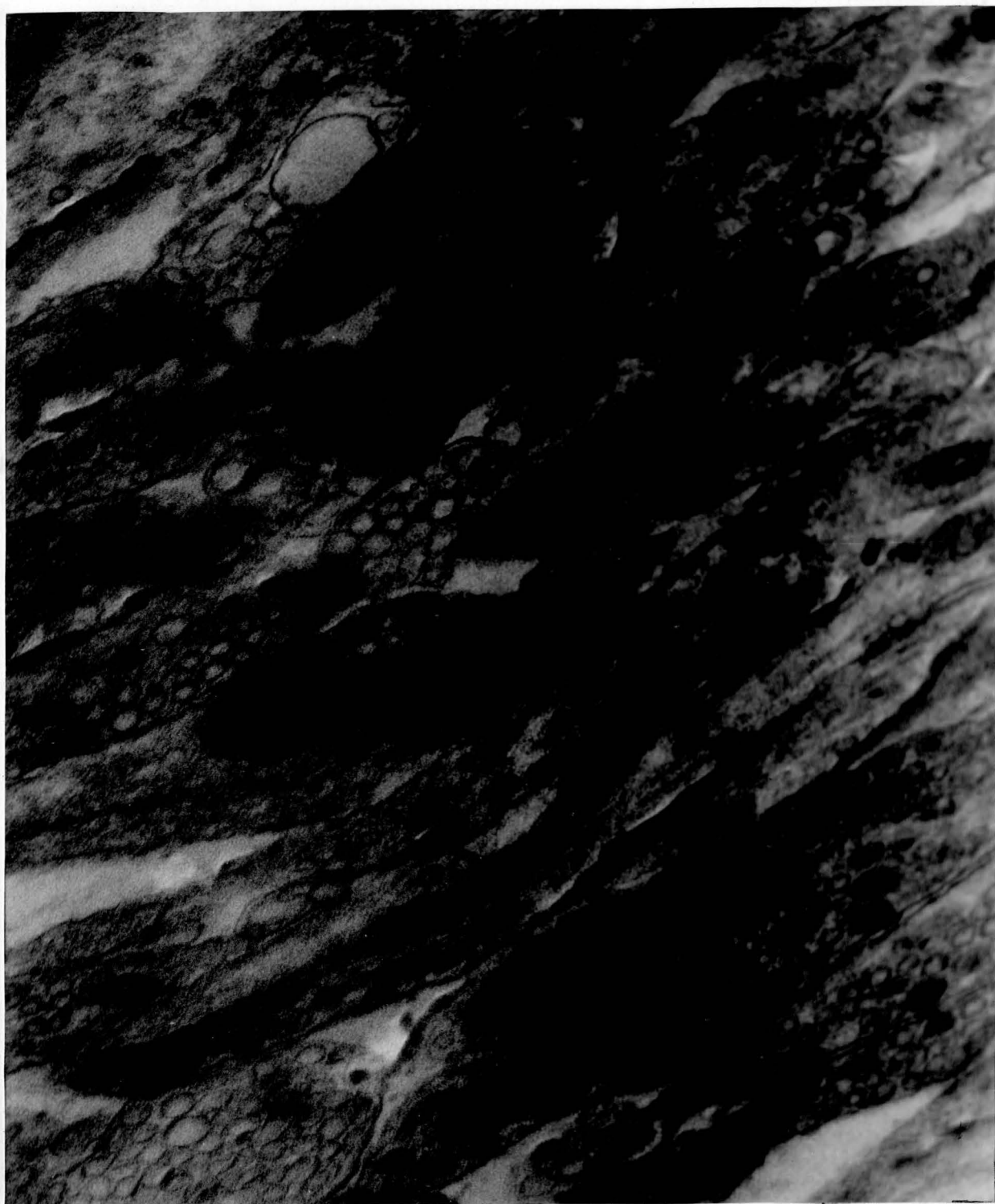


FIGURE 23

PLATE 17

EXPLANATION OF FIGURE

Figure 24: Two ganglion cells in the 26 day postoperative tissue. The cytoplasm is filled with a large amount of free ribosomes and the network of filaments in the upper cell is still well developed. Notice however that the number of mitochondria has decreased and several of them are misshapen. X32,000.

F = Filaments
M = Mitochondrion
N = Nucleus
RNP = Ribonucleoprotein (ribosomes)

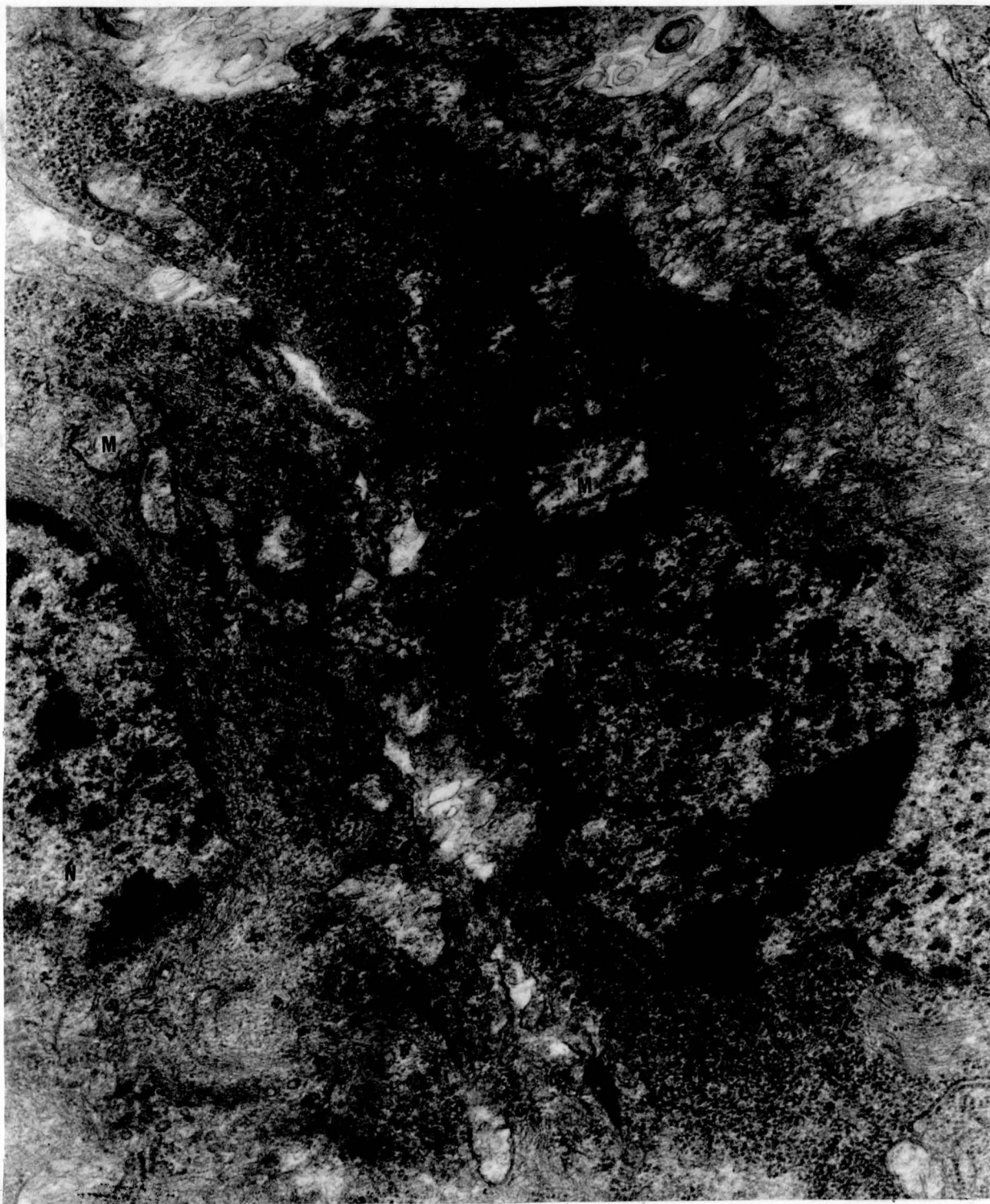


FIGURE 24

PLATE 18

EXPLANATION OF FIGURE

Figure 25: A cell in the outer portion of the 26 day suprapharyngeal ganglion. The cytoplasm of this cell contains many RNP particles in rosette configurations. Notice that the cell processes envelop an area of amorphous ground substance that contains the debris of dead cells. X40,000.

N = Nucleus

RNP = Ribonucleoprotein (ribosomes)



FIGURE 25

PLATE 19

EXPLANATION OF FIGURE

Figure 26: Cytoplasm of a 34 day ganglion cell. Notice the elaborate matrix of agranular endoplasmic reticulum. The distal portions of the reticulum are fragmented and the membranes envelop a portion of electron dense material. Several elementary visicles (700Å) are located near the reticulum. There are also free ribosomes scattered throughout the cytoplasm although many of them are aligned against the walls of short cisterns.

AER = Agranular endoplasmic reticulum
N = Nucleus



FIGURE 26

PLATE 20

EXPLANATION OF FIGURE

Figure 27: A terminal branch of the prostomial nerve in the cord sheath near the surface of the 34 day ganglion. There are many axon terminals filled with vesicles, primarily the clear or the dark "halo" type. Notice the glial processes that invest several nerves and separate them from the collagen of the sheath. X32,000.

Ax = Axon
Col = Collagen
Gl P = Glial process



FIGURE 27

APPROVAL SHEET

The thesis submitted by Stephen Christopher Moore has been read and approved by the following Committee:

Dr. Robert Hadek, D.V.M., Ph.D.

Dr. Leslie A. Emmert, Ph.D.

Dr. Joel Brumlik, M.D.

The final copies have been examined by the chairman of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

01/07/76
Date

Robert Hadek
Director's Signature